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JACQUES LOEB

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THE EFFECT OF RADIOACTIVE RADIATIONS AND X-RAYS ON ENZYMES.

II. THE EFFECT OF RADIATIONS FROM RADIUM EMANATION ON PEPSIN IN SOLUTION.

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(Received for publication, June 12, 1923.)

In a previous communication¹ we have reported observations on some aspects of a radiochemical reaction, effected by the radiations (beta and gamma) from radium emanation and its radioactive products in equilibrium with it, in which the enzyme trypsin is inactivated. Our experiments brought out the significant fact that *under definite conditions of irradiation*, the change in the logarithm of the concentration of active trypsin is a linear function of a variable, W , which is algebraically equal to the product of the average amount of radium emanation present, E_a , and the time of exposure, t ; i.e., $E_a t = W$.² The experimental results obtained are formulated in the following equation.

$$\log_e Q - \log_e Q_0 = -kW \quad (1)$$

Where Q_0 is the initial concentration of active trypsin and Q is the concentration of active trypsin found after the solution has been

¹ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1922-23, v, 647.

² As explained in a communication immediately following this one, W represents the amount of energy liberated by the radioactive source during a period of exposure and should therefore be measured in energy units. In order to give clear definition to the variables involved in the experiments we have introduced a unit of measure of activity P which we have named the *curie-power*. The amount of emanation in equilibrium and the activity of the preparation are numerically equal, therefore the relation $E_d = P_d = W$ exists, and W is measured in terms of the energy unit *curie-power hour*.

irradiated for a given value of W . The concentration of the enzyme is expressed in arbitrary units. When this equation is solved for k we obtain

$$k = \frac{1}{W} \log_e \frac{Q_0}{Q} \quad (2)$$

We have found that pepsin in solution is also inactivated by the radiations discussed. The results of our experiments indicate that the law expressing the quantitative relation between the variables, Q and W , when trypsin solutions are irradiated holds satisfactorily for pepsin solutions too. The data obtained from observations made following the exposures of 4.6 cc. of a 1 per cent solution of pepsin (Fairchild's) to the radiations (beta and gamma) from radium emanation for the "millicurie hour periods" indicated were as follows:

Millicurie hours W	Units of active pepsin found. Q	$k = \frac{1}{W} \log_e \frac{Q_0}{Q}$ $k \times 10^3$
	$Q_0 = 1.30$	
2662	1.12	5.61
5900	0.950	5.35
9810	0.776	5.25

The value for Q_0 is the average of six observations and the values given for Q are the average of two.

The graphic presentation of the results is given in Fig. 1, where it will be observed that $\log Q$ is a linear function of the variable, W , as is required to satisfy equation (1).

Technical Procedure.

The pepsin solution was prepared as follows: 5 gm. of Fairchild's powdered pepsin were scattered over the surface of 100 cc. of 0.06 M hydrochloric acid contained in a 500 cc. pyrex glass beaker. After standing overnight at room temperature, the solution was stirred and poured into a pyrex glass flask. This flask with the pepsin solution was then placed in a water bath for 24 hours. The temperature of the bath was maintained at $34^\circ \pm 0.02^\circ\text{C}$. One part of the resulting solution mixed with four parts of distilled water was the

dilution used in the test. This solution was of about pH 4.2, as determined colorimetrically, and 0.5 cc. contained 1.30 units of active pepsin.

The activity of the pepsin in solution was determined by Northrop's viscosity method.³ The gelatin solution employed was prepared by adding 25 cc. of $M/30$ H_3PO_4 to 975 cc. of an aqueous solution of isoelectric gelatin containing a sufficient amount of gelatin to make the final preparation 3 per cent gelatin by dry weight. This solution had a pH of about 4.4, and about 185 seconds were required for 5 cc. to pass through the viscosity pipette.

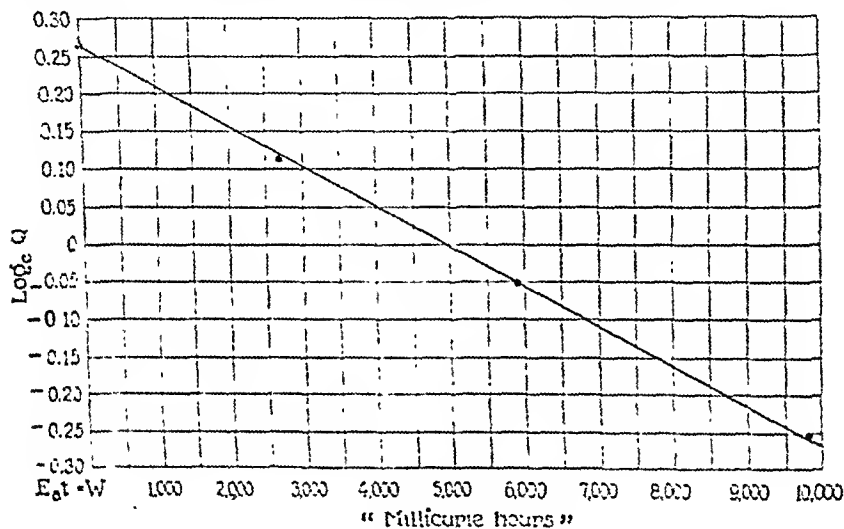


FIG. 1.

The arrangement for irradiating the pepsin solutions differed somewhat from that stated for our previous investigation with trypsin. The radium emanation was contained in a spherical glass bulb which was fused to a length of fine glass rod. The outside diameter of the bulb was approximately 3 mm. The wall was sufficiently thick to prevent the passage of α radiations, but interfered only slightly with the passage of β and γ radiations. In all the tests the pepsin solution was put into a spherical bulb of 4.6 cc. volume and

³ Northrop, J. H., and Hussey, R. G., *J. Gen. Physiol.*, 1922-23, v, 353.

the small bulb containing the emanation was placed as near the center of the solution as possible. The thickness of the absorbing layer for the radiations emitted from the radium emanation was approximately 9.6 mm. This arrangement permitted the absorption of practically all the β radiations emitted. The bulb containing the test was kept in melting ice during each exposure.

DISCUSSION.

It is of interest to note that when the same volume of dilute trypsin solutions is irradiated under the conditions described above for pepsin, we find that the rate of the reaction is approximately twenty times as great. In addition to fundamental concepts involved in discussing the variations in the speed of chemical reactions in general, there are other factors to consider in comparing the difference observed in the speeds of these two radiochemical reactions. For example, in both of these chemical systems, we have different amounts of foreign material which go into solution with the enzyme. Necessarily this material absorbs a certain amount of energy which will not be utilized in effecting the chemical change under consideration. In the pepsin solution, the amount of this material is much greater than in the trypsin solution. We have to further regard possible differences in the effect of the radiations on the enzyme inhibitor compound in the two solutions. It seems at this time not improbable that experiments can be made which will throw some light on what bearing these factors have on the issue.

Whether this law of radiochemical change has wider application remains to be determined by similar experiments with other chemical systems. If this is found to be the case it would appear that we have a possible method which will permit a quantitative determination of the relative effect of various forms of radiant energy. Further investigations of these radiochemical reactions may lead to the development of a basis for the interpretation of the reactions effected in living matter by these forms of radiant energy.

CONCLUSION.

Pepsin in solution is inactivated by the radiations (beta and gamma) from radium emanation. This chemical effect has been studied quantitatively.

The principles involved in this radiochemical reaction are apparently the same as those found in the case of trypsin previously reported; namely, the change in the logarithm of the concentration of active enzyme varies directly with the variable, *W*.



THE EFFECT OF RADIOACTIVE RADIATIONS AND X-RAYS ON ENZYMES.

III. A UNIT OF MEASURE OF ACTIVITY FOR RADIUM EMANATION.

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(Received for publication, July 30, 1923.)

In our studies on the radiochemical reaction effected by the radiations (beta and gamma) from radium emanation in which enzymes are inactivated we have stated that in a given system the chemical change is a function of the product of two variables; namely, the average amount of emanation present, E_a , and time, t . The product of these two variables has been equated to a single variable, W , for which we have employed the expression "millicurie hours" as a unit of measure. This is not, however, a suitable unit of measure of the quantity of energy which W represents. That no other expression has been available is due to the circumstance that up to the present time but little attention has been given to making clear a distinction between activity and amount of radium emanation. The curie is defined as the amount of emanation in equilibrium with 1 gm. of radium element. In our experiments we are concerned not only with the amount of emanation but more particularly with the *activity* or *power*¹ of the preparation, for which no unit has yet been assigned. In this communication we shall endeavor to show the desirability of having such a unit and suggest what appears to us to be one that will meet our

¹ We use *activity* and *power* synonymously in the sense in which the words are defined in physics. Cf. Duff, A. W., A text-book of physics, Philadelphia, 1916, 4th edition, 44. It is necessary that we emphasize this definition since activity is sometimes used in a descriptive sense in discussions on radioactive radiations. Thus, activity is used to denote the "intensity" of electrical, or other effect, of the radiations from one radioactive substance compared to another.

requirements. A simple means of developing a concept for this unit is suggested by the experimental fact that the *activity* of any given preparation of radium emanation is proportional to the amount of emanation present. Indeed, the measurement of *amount* of radium emanation is based on this fact.

Energy is liberated from radium emanation and its radioactive products in equilibrium with it as kinetic energy; contributed by four principal components; namely, alpha, beta, and gamma radiations and recoil atoms. The *rate* of liberation of this energy has been measured by Rutherford by means of converting the kinetic energy of these components into heat. From observations made in an experimental arrangement which permitted nearly complete absorption of the radiations emitted from 1 curie of radium emanation and its radioactive products in equilibrium with it, Rutherford² found the rate of liberation of energy expressed in heat units, to be approximately 109.3 gram calories per hour. *This rate of liberation of energy by the above mentioned source we propose to define as a unit of power or activity of such a radioactive source.* As a name for this unit we suggest the expression *curie-power*. Accordingly the *power* or *activity*, which we shall denote as P , of a given preparation of radium emanation, expressed in terms of the unit *curie-power*,³ is numerically equal to the amount of emanation present, expressed in curies; *i.e.*, the variables, E and P , are algebraically equal. We can express the relation of the variables considered as follows:

$$E_a t = P_a t \equiv W \quad (1)$$

in which E_a is expressed in units of mass, curies; P_a is expressed in units of *activity*, the *curie-power*; t is time expressed in hours in each case. Then W , representing a quantity of energy, is identical with the product $P_a t$, *i.e.* power and time, is expressed in energy units

² Rutherford, E., *Radioactive substances and their radiations*, Cambridge, 1913, 580.

³ The unit, *curie-power*, will have the same significance in radium emanation measurements that the unit, *candle-power*, has in illumination measurements. The appropriateness of this name may be questioned but at the present time it appears to us the most fitting one we can suggest. For practical purposes the *millicurie-power* is used for convenience.

as it should be. Since the *unit of activity* is the *curie-power*, the *energy unit* will be designated the *curie-power hour* and 1 *curie-power hour* is equal to 109.3 gram calories.

We shall now consider the principles involved in the determination of the quantity of radium emanation and indicate that the determination is based on the measure of *relative activity* or *power*. For example, amounts of emanation are determined by comparing the ionizing effect of the gamma radiations emitted by a source of unknown value with the effect of the same kind of radiations emitted by a standard source containing emanation in equilibrium with a known amount of radium element. The ionization is effected in air contained in an ionization chamber and the measurements are made under fixed conditions of observation. When ionization of a gas is effected by these radiations in an electric field where the potential gradient is sufficiently great to practically prevent the recombination of ions produced, the electric current which flows is called the saturation current. The magnitude of this current depends upon the number of pairs of ions produced per unit time and its value is the measure of the ionizing effect of the radiations. It is therefore a measure of the *rate of production of ions*. Since the production of ions depends upon the *ability* of the radiations *to do work* it follows that the number of pairs of ions produced per unit time depends upon *the amount of work done per unit time* by the radiations. Clearly then, in a given system, the rate of production of ions varies with the *power* or *activity* of the radioactive source. When the value for the *relative activity* of two preparations is desired, as in the case of radium emanation measurements, it is necessary that the ionization be effected by radiations of the same kind or quality.

The use of the units suggested above permits us to make a more comprehensive statement of our experimental results already published.^{4,5} The relation which was found to exist between the chemical change observed and the variable, W , is stated by the equation

$$\log Q - \log Q_0 = -kW \quad (2)$$

⁴ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1922-23, v, 647.

⁵ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1923-24, vi, 1.

where the logarithms are to the base e ; Q_0 is the initial concentration of active enzyme expressed in arbitrary units; Q is the concentration of active enzyme found following an exposure of the enzyme solution to the radiations discussed, for a given increment of energy, W .⁶ W is expressed in *millicurie-power hours*. From equation (1) we can write

$$W = P_0 t = E_0 t$$

Now

$$E_0 t = \int_0^t E dt = E_0 \int_0^t e^{-kt} dt$$

Since

$$E = P, P_0 = E_0$$

Whence

$$W = \int_0^t P dt = P_0 \int_0^t e^{-kt} dt = P_0 t \quad (3)$$

In any experiment Q_0 is constant, therefore equation (2) may be written

$$\log Q = -kW + C$$

which on differentiation becomes

$$\frac{1}{Q} \frac{dW}{dQ} = -k, \quad \text{or} \quad dQ = -k Q dW \quad (4)$$

from equation (3) it is evident that $P dt = dW$ and if we substitute this value of dW in equation (4) we can write the differential equation for the entire experiment; i.e.,

$$dQ = -k Q P dt \quad (5)$$

⁶ It is evident from the preceding discussion that W represents the total energy liberated by the radioactive source during the period of exposure. According to Rutherford's data referred to, the energy contributed per hour per curie by the different components, expressed in gram calories, is as follows: alpha radiations and recoil atoms, 98.5; beta radiations, 4.3; and gamma radiations 6.5; total 109.3. In the arrangement employed for our experiments the alpha radiations and recoil atoms cannot penetrate the walls of the container in which the emanation is confined. Some low velocity beta radiations also fail to penetrate the walls of the container. In any experiment then we have available less than 10.8 gram calories per hour, per curie. Unfortunately, in the paper referred to in foot-note 4 these values are not correctly stated.

Hence it follows that the time rate of change in the concentration of active enzyme is proportional to the concentration of the active enzyme and the *activity* or *power* of the radium emanation.

CONCLUSION.

In this communication we have introduced a unit to express *activity* or *power* of a given preparation of radium emanation. We have named this unit the *curic-power* and defined it as *the activity of 1 curie of radium emanation and its radioactive products in equilibrium with it*. We suggest the introduction of this unit in order that we may make a more comprehensive statement of our experimental observation.

In the radiochemical reaction effected by the radiations (beta and gamma) from radium emanation in which enzymes are inactivated the chemical change in a given system is a function of the product of two variables; namely, the average activity of the radium emanation, P_a , expressed in terms of the unit *millicurie-power*, and time, t , expressed in hours. This product has the dimensions of energy and is identical with W which is measured in terms of the energy unit, *millicurie-power hours*.

THE MINIMUM CONCENTRATION OF OXYGEN FOR LUMINESCENCE BY LUMINOUS BACTERIA.

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(Received for publication, July 9, 1923.)

It has been recognized for a long time that luminous organisms require very little oxygen for light production, so little in fact that some observers have declared the luminescence to occur without oxygen.¹ Although it is sometimes difficult to free a solution of a luminescent substance from the last traces of oxygen, and this fact is responsible for the controversy, it is safe to say that all bioluminescence is dependent on dissolved oxygen.²

This statement is easily verified, as far as luminous bacteria are concerned, by allowing an emulsion of luminous bacteria in sea water to stand in a test-tube. In a short time, depending on the number of bacteria present, the tube, formerly glowing throughout, becomes perfectly dark except at the surface in contact with air. The bacteria have used up all the oxygen in the sea water. Beijerinck³ employed this method to demonstrate the activity of chloroplasts in decomposing CO₂, apart from the living plant cell. A mixture of luminous bacteria and thoroughly ground clover leaves containing suspended chloroplasts was allowed to stand in perfect darkness until all the oxygen dissolved in the emulsion had been used up, and the tube became dark. If a match were now lighted, the light, striking the chloroplasts, caused them to decompose CO₂, as during photosynthesis, with consequent liberation of oxygen. The oxygen formed then caused the luminous bacteria to glow for a short time, until again used up.

In the above experiment we employ luminous bacteria as a test for oxygen and it is proper to inquire what concentration of oxygen

¹ Kanda, S., *Ann. J. Physiol.*, 1919-20, i, 544.

² Harvey, E. N., *Ann. J. Physiol.*, 1920, ii, 580.

³ Beijerinck, M. W., *Ned. akad. van wetensch. te Amsterdam*, 1902, iv, 45.

can be detected in this way. It is quite obvious that in any determination of such a small concentration of oxygen, (1) the emulsion of luminous bacteria must be continually maintained in equilibrium with some inert gas containing so small a percentage of oxygen that a just perceptible light is produced; (2) the concentration of luminous bacteria must be sufficiently small so that the continuous utilization of oxygen by the bacteria does not disturb the equilibrium. These two conditions have been realized in the experiments described in this paper. The continual maintenance of equilibrium is easily obtained by rapid bubbling of the gas mixture through the bacterial emulsion which was well below the concentration, as determined by experiment, at which an appreciable amount of oxygen was used.

The method of mixing gases by means of flow-meters, so widely used during the war, was employed. The apparatus is shown in Fig. 1. From a tank of electrolytic hydrogen, hydrogen gas, containing 0.0034 by volume oxygen (as determined by analysis), passed through a Y-tube to two flow-meters, *A* and *B*. The gas which passed through the *A* flow-meter, giving a rapid flow, had all of its oxygen removed by passing through a quartz tube, *C*, over platinized asbestos⁴ heated to dull redness, and was absolutely pure hydrogen,⁵ so far as oxygen was concerned. The gas passing the *B* flow-meter, which contained a very fine capillary, and hence a slow flow of gas, contained the same amount of oxygen as that in the tank, 0.0034. The gases from *A* and *B* were mixed at the three-way cock, *D*, and passed through the bacterial emulsion in the tube, *E*. A safety trap at *F* and needle valves at the tank and at *G* to regulate the flow of gas, completed the arrangements. All parts of the apparatus train were glass joints or connections of lead tubing with de Khotinsky cement, the only rubber connection being a thick short tube between *D* and *E*, and another making connection with the hydrogen tank *before* the hydrogen passed over the platinized asbestos. It is

⁴ In some experiments a platinum spiral heated red hot by passing an electric current through it was substituted for the platinized asbestos. Both gave efficient oxygen removal.

⁵ Dr. H. S. Taylor of Princeton University has very kindly calculated for us that hydrogen so treated would contain oxygen at a pressure of 2×10^{-26} atmospheres, a negligible quantity.

not possible to keep pure hydrogen free of oxygen if it is carried through any distance of rubber tubing. In order to prevent contamination of pure hydrogen with oxygen dissolved in the manometer fluids the *A* flow-meter manometer, after careful washing out with pure hydrogen, was filled with alkaline pyrogallol solution from *H*, while the *B* flow-meter manometer contained water in equilibrium with hydrogen containing 0.0034 oxygen, filled from *J*.

Once a flow-meter has been calibrated for a given gas in terms of the difference in pressure at the two ends of the capillary, it can be used at any time for the same gas and with the same manometer fluid. Calibration of *A* and *B* was effected by determining the time necessary for a given volume of hydrogen gas to pass through when the difference in pressure at the ends of the capillary was varied, and a curve drawn relating cubic centimeter flow per second to pressure difference. Rate of flow through *A* varied in the range of pressure differences actually used from 2.53 cc. per second at 14 cm. to 3.17 cc. per second at 18 cm. alkaline pyrogallol solution. Rate of flow through *B* varied from 0.006055 cc. per second at 24 mm. to 0.007888 cc. per second at 32 mm. water.

The percentage of oxygen in the hydrogen was determined by passing the gas through a long tube of CaCl_2 to dry it, then over hot platinized asbestos which formed water, by combination of any oxygen with the hydrogen. The gas then passed through two small U-tubes filled with CaCl_2 to collect the water formed, and was measured over water in a collecting flask. The second CaCl_2 U-tube was not necessary as weighings showed no increase in weight. All the water was absorbed by the first tube. The second tube served as a control. 2400 cc. of gas at 18°C . and 767 mm. Hg gave 0.0122 gm. water, an average of three determinations within 0.1 mg. of each other. Reduced to 0°C . and 760 mm. pressure and corrected for the vapor tension of water at 18°C ., 2227 cc. of hydrogen contained 7.589 cc. of oxygen at 0°C . and 760 mm. or 0.0034, 0.34 per cent.

An actual determination of the oxygen necessary for luminescence is run somewhat as follows: After flushing out the whole apparatus with tank hydrogen through its various exits, the quartz tube, *C*, with its platinized asbestos is heated and *A*, as well as vessel *H*,

containing alkaline pyrogallol, flushed out with oxygen-free hydrogen. The alkaline pyrogallol is then allowed to flow into the arms of the manometer tube. The water in *J* is then brought into equilibrium with tank hydrogen by shaking and refilling with tank hydrogen several times and then allowed to flow into the manometer of the *B* flow-meter. The emulsion of luminous bacteria⁶ in sea water is placed in *E* which is then attached to the three-way cock, *D*, so turned that pure hydrogen gas alone passes through the bacteria. The flow is regulated by turning the needle valve at the hydrogen tank to give a flow of gas, corresponding to a difference in level of about 16.7 cm. alkaline pyrogallol solution. With needle valve, *G*, shut so that no flow of gas passes the *B* flow-meter, the bacteria in *E* are absolutely dark. Observations must be made in a black box in a dark room to prevent confusion from reflected light, and the eyes perfectly dark adapted. Upon now opening needle valve, *G* very slowly to admit more and more tank hydrogen and hence a little oxygen, the bacteria just begin to glow at approximately 25 mm.⁷ water difference of pressure of *B*, corresponding to 0.00630 cc. per second. The level in the *A* flow-meter will have changed slightly perhaps to 16.6 cm., corresponding to 3.00847 cc. per second. There is thus passing through the bacterial emulsion 3.01477 cc. of gas each second, of which $\frac{0.00630}{3.01477}$ is supplied by the *B* flow-meter and of this gas supplied by the *B* flow-meter 0.0034, or 0.34 per cent, is oxygen. A simple calculation shows that in the gas flowing through the luminous bacteria there is 0.0000071 oxygen, or 0.0007 per cent. This corresponds to one part oxygen in 143,000 parts hydrogen by volume, or 0.0053 mm. Hg oxygen pressure. Assuming that Henry's law holds, since 1 cc. of sea water dissolves about 0.027 cc. of oxygen⁸ at 20°C. and 760 mm. Hg there will be present in each cubic centimeter of the sea water containing luminous bacteria glowing very

⁶ The luminous bacteria were isolated from fish obtained at the fish store in Princeton and grown in sea water containing 1 per cent Bacto-peptone, 1 per cent glycerol, some beef extract, and adjusted to a pH of 7.7.

⁷ Average of eight determinations.

⁸ Fox, *Tr. Farad. Soc.* 1909, v, 77.

faintly at 0.0053 mm. Hg oxygen pressure, 0.00000019 cc. of oxygen or about 0.0000000027 gm. of oxygen, or 1 part oxygen by weight in 3,700,000,000 cc. of sea water.

2 mm. change of level in *B* downward from 25 to 23 mm. Hg will cause the light in *E* to disappear, while 2 mm. change of level of *B* upward from 25 to 27 mm. Hg, corresponding to 0.00677 cc. per second, giving the bacteria 0.0000076 oxygen, will cause an unmistakably brighter light to appear in *E*. Hence the method is sensitive to 5 parts in 76 or about 6 per cent and the greatest error is no doubt the variation in sensitiveness of the dark adapted eye or variations in the light of the bacteria. A number of the values

TABLE I.

Readings of Flow-meters When Bacteria Give Just Perceptible Light.

Experiment No.	A manometer.	Flow through A per sec.	B manometer.	Flow through B per sec.
	mm.	cc.	mm.	cc.
1	148	2.689	23	0.00583
2	145	2.643	23	0.00583
3*	145	2.643	28	0.00700
4	176	3.141	21	} = 24.6 0.00621
5	176	3.141	24	
6	176	3.141	24	
7	176	3.141	28	
8	176	3.141	26	

* This determination works out to 0.0000089 oxygen.

obtained at different times with the same or with different emulsions of luminous bacteria are shown in Table I.

An experiment to show the values obtained with different concentrations of bacterial emulsions. run the same evening on the same bacteria is given in Table II. C represents a fairly concentrated bacterial emulsion which was diluted with sea water to give C/20, C/30, etc. It will be noted that with concentrated bacterial emulsions more oxygen is necessary to give a just visible luminescence, as shown by the values of the *B* flow-meter, because the bacteria are present in sufficient quantity to use up some of that supplied. However, from a dilution of C/30 to C/1,000 we have practically con-

stant readings for *B* which indicates that concentration of bacteria is playing no part in these determinations. The last eight values given in the table are the ones averaged for calculation of the value 0.000007 oxygen.

TABLE II.

Readings of Flow-meters When Bacteria of Varied Concentrations Give Just Perceptible Light.

Concentration of bacterial emulsion.	A manometer.	Flow through A per sec.	B manometer.	Flow through B per sec.
	mm.	cc.	mm.	cc.
C	$\begin{cases} 165 \\ 165 \end{cases}$	$\begin{cases} 2.983 \\ 2.983 \end{cases}$	$\begin{cases} 47 \\ 49 \end{cases} = 48$	0.01130
C/20	$\begin{cases} 165 \\ 165 \end{cases}$	$\begin{cases} 2.983 \\ 2.983 \end{cases}$	$\begin{cases} 31 \\ 31 \end{cases} = 31$	0.00722
C/30	$\begin{cases} 165 \\ 165 \\ 166 \\ 166 \end{cases}$	$\begin{cases} 2.983 \\ 2.983 \\ 3.008 \\ 3.008 \end{cases}$	$\begin{cases} 24 \\ 26 \\ 24 \\ 25 \end{cases} = 25$	0.00630
C/100	$\begin{cases} 166 \\ 166 \end{cases}$	$\begin{cases} 3.008 \\ 3.008 \end{cases}$	$\begin{cases} 26 \\ 24 \end{cases} = 25$	0.00630
C/500	$\begin{cases} 166 \\ 166 \end{cases}$	$\begin{cases} 3.008 \\ 3.008 \end{cases}$	$\begin{cases} 26 \\ 25 \end{cases}$	
C/1,000	$\begin{cases} 166 \\ 166 \end{cases}$	$\begin{cases} 3.008 \\ 3.008 \end{cases}$	$\begin{cases} 26 \\ 24 \end{cases}$	

SUMMARY.

A method is described for measuring the concentration of oxygen to allow just perceptible luminescence of luminous bacteria. The value turns out to be extraordinarily low, about 0.005 mm. Hg pressure O₂ or 1 part by weight oxygen dissolved in 3,700,000,000 cc. sea water.

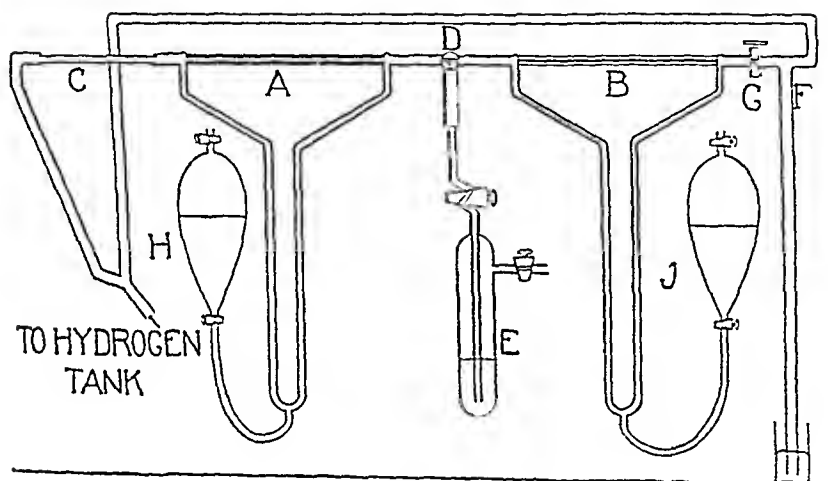


FIG. 1.

THE RATE OF GROWTH OF THE DAIRY COW.

III. THE RELATION BETWEEN GROWTH IN WEIGHT AND INCREASE OF MILK SECRETION WITH AGE.

By SAMUEL BRODY, ARTHUR C. RAGSDALE, AND CHARLES W. TURNER.
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(Received for publication, June 29, 1923.)

In a preceding communication¹ it was shown that after the age of 2 years, the rate of growth of the dairy cow declines in a non-cyclic manner, and that the course of decline in the rate of growth follows the course of decline in the rate of a monomolecular chemical reaction. Since increase in weight may be due to the storage of inert substances rather than to increase in the mass of living tissues, it is desirable to substantiate the weight data by other measurements. In this communication we report on the change of a physiological activity with age—that of milk secretion—and show that the increase of milk secretion with age follows the same course as the increase of body weight with age, and that while milk secretion and body weight follow the same course, they are largely independent of each other.

If the increase of milk secretion with age follows the same course as the increase of body weight with age then there should be a linear or directly proportional relation between milk secretion and body weight for a group of animals of all ages during the growing period. This is, in fact, the case as shown in Fig. 1 plotted from the data in Table I. The data is confined to the age interval of from 2 years, the usual age when milking begins, to 9 years, the age of maximum milk secretion and body weight of the dairy cow.

It is of interest to fit an equation to the data in Fig. 1 in order to determine whether the relation between body weight and milk secretion with age is an expression of a general law which will apply below the range of observation of 2 years. Fitting an equation of the first degree to the data, we obtain

$$F = 1.0425W - 472.32$$

¹ Brody, S., Ragdale, A. C., and Turner, C. W. *J. Gen. Physiol.*, 1922-23, v, 445.

in which F is the yearly fat production for any body weight, W . That is, after the animal reaches the body weight of 472 pounds there is an increase of 1.0425 pounds in milk fat production per year, for an increase of one pound of body weight with age. Since the animal is about 13 months of age when she weighs 472 pounds,² then if this

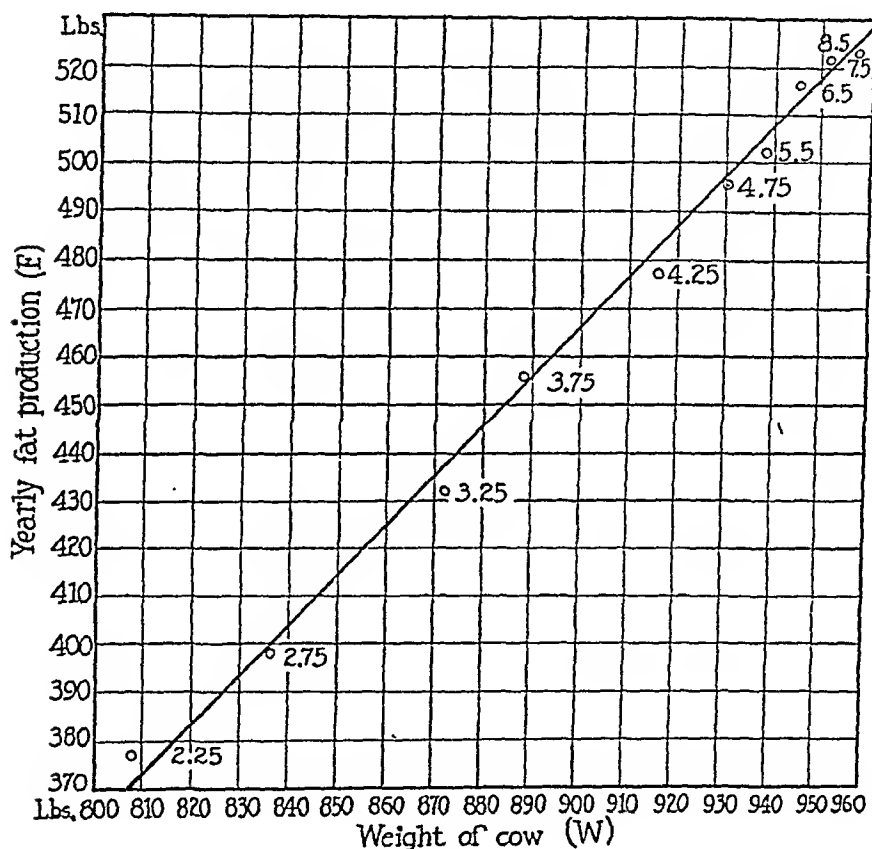


FIG. 1. The increase of milk secretion with increasing body weight with age in Jersey cattle. The smooth line passing through the observed values was plotted from the equation $F = 1.0423W - 472.32$ in which F is the yearly milk fat production for any body weight (W). From this equation an increase of 100 pounds in the weight of the body with age is accompanied by an increase of about 104 pounds of milk fat per year. The numerals on the curve represent the ages of the animals in years.

² Cf. Eckles, C. H., *Univ. Missouri Agric. Exp. Station, Research Bull.* 36, 1920.

equation is an expression of a general law, the dairy cow should be able to begin secreting milk at a little over one year of age. This is in agreement with experience. Calves have been known to conceive as early as 5 months of age, and to calve at 14 months of age. This equation therefore appears to be rational, and incidentally seems to indicate that there is no material development of mammary milk secreting tissue until the age of sexual maturity, or at any rate, until the age of adolescence is reached.

TABLE I.

*The Increase of Body Weight and Milk Secretion with Age in the Dairy Cow.**

Age.	Body weight.		Butter fat production per year.										
	Jersey.		Jersey.		Shorthorn.		Ayrshire.		Guernsey.		Holstein		
	No. of cows included.	Average weight.	No. of cows included.	Average production.	No. of cows included.	Average production.	No. of cows included.	Average production.	No. of cows included.	Average production.	Age.	No. of cows included.	Average production.
	yrs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	yrs.	lbs.	lbs.
2.25	3155	808	2829	377	123	268	817	333	3559	407	2.5	2454	508
2.75	1449	836	1261	398	183	274	893	366	1682	423	3.5	1523	570
3.25	1523	872	1271	432	82	251	442	367	1374	436	4.5	1238	615
3.75	1122	888	992	456	85	317	461	389	1192	472	5.5	1116	644
4.25	1171	916	978	477	61	348	376	408	1093	486	6.5	836	666
4.75	916	930	809	496	64	356	340	430	884	501	7.5	583	665
5.5	1692	938	1487	502	70	376	545	448	1153	511	8.5	396	657
6.5	1235	945	1067	516	80	383	399	455	897	521			
7.5	965	952	857	521	66	405	298	478	572	528			
8.5	621	957	565	523	65	402	225	453	369	527			

* Compiled from the records of Register of Merit Jersey, Record of Merit Shorthorn, Advanced Register Ayrshire, Guernsey and Holstein cattle.

While the linear relation between increase in body weight and milk secretion with age in Fig. 1 definitely shows that growth in body weight and increase in milk secretion with age follow the same course, it is useful to bring out this relation by pointing to the parallelism between the two processes. In Fig. 2 the curves of increase in body weight (broken curve) and milk secretion (continuous curve) of the Jersey cow are parallel and both follow the same exponential equation

$$X = A(1 - e^{-kt})$$

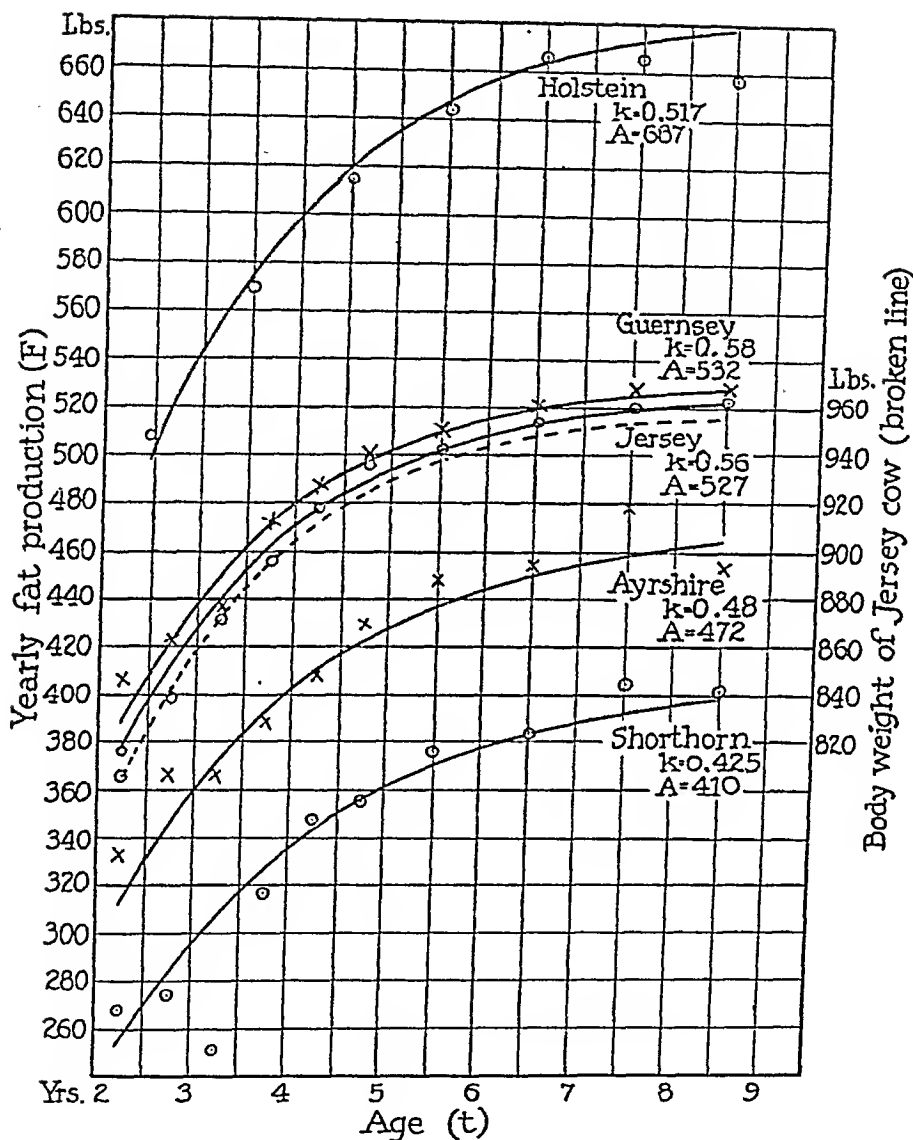


FIG. 2. The increase of milk secretion with age in the dairy cow. The smooth curves passing through the observed values were plotted from the equation $F = A(1 - e^{-kt})$ in which F is the yearly fat production at any age (t). The numerical values of the constants A and k for the several breeds are indicated on the curves. The broken curve shows the growth in weight of the Jersey cow.

where X is the body weight, or milk secretion at any age, t , and k is the velocity constant which is of approximately the same numerical value for both curves. This equation also represents the course of milk secretion of other breeds of cattle as shown by the close agreement between the values computed from this equation represented by the smooth curves in Fig. 2, and the observed values.

The data in Figs. 1 and 2 show conclusively that growth in weight, and increase of milk secretion with age follow the same course—the course of a monomolecular chemical reaction—thus further substantiating the theory that growth is limited by a chemical reaction.³

³ The decline in growth with advancing age is often spoken of as due to the dying out of the "growth impulse." From the standpoint of the quantitative interpretation of this equation, the concept of growth impulse may be used in the same sense as the concept of "limiting substance." This equation shows that the growth impulse dies out, or the growth-limiting substance is used up at a constant ratio (or per cent) of itself. This idea is illustrated in the following table on the growth in weight of the Jersey cow, assuming that the mature weight, 960 pounds, is a measure of the limiting substance at the beginning of growth, and that the weight at any age t is a measure of this limiting substance used up to that age t .

Age. (t)	Weight of Jersey cow. (X)	Amount of limiting substance or impulse left, or growth yet to be made. ($960-X$)	Ratio of each value of ($960-X$) to the preceding value of ($960-X$).
yr.	lbs.		
1.75	748	212	
2.25	803	157	0.740
2.75	844	116	0.738
3.25	875	85	0.732
3.75	896	64	0.752
4.25	913	47	0.734
4.75	925	35	0.744

The amount of limiting substance or impulse, at each age is seen to be about 74 per cent of the amount of this substance, or impulse, at the preceding age. In other words, the proportional amount of limiting growth substance, or of growth impulse, that changes in unit time is a constant.

Having determined the fact that the increase of milk secretion with age follows the same course as the increase of body weight with age, the questions arise whether the increase of milk secretion with age is dependent on the increase of body weight (*i.e.*, whether the increase of milk secretion with age is limited by the increase of nutrients circulating in the blood which are roughly proportional to the body weight); or whether the increase of milk secretion with age is dependent on the development of milk secreting tissues, and on other factors consequent to increase in age. These questions may be answered in a general way by separating, as far as practicable, the factors of age and body weight, and determining the effect of each of these factors on milk secretion. A practical, if not an entirely satisfactory⁴ method of making a separation between body weight and age is to classify the animals into convenient age groups and determine the relation between the body weight of the animals within the age groups and their milk secretion; also to classify the animals into convenient body weight groups and determine the relation between the age of the animals within the weight groups and their milk secretion. This has been done and the results are shown in Fig. 3 plotted from data in Tables II and III.

Fig. 3 shows the relation between milk secretion and body weight at constant age. Qualitatively, the effect of age is apparent by the fact that each age group has its own curve which is not connected to the curves of other age groups. Quantitatively, the relative contribution of increasing body weight and of age (or rather of factors other than body weight incident to increase of age) on increasing milk secretion with age, may be roughly estimated from a comparison of the slopes of the curves of Figs. 1 and 3. From Fig. 1, showing the relation between milk secretion and body weight with age, an increase in body weight with age by 100 pounds is accompanied by an increase in yearly butter fat production of slightly over 100 pounds. From Fig. 3 showing the relation between body weight and milk secretion at constant age,

⁴ This method is not entirely satisfactory because differences in body weight at the same age may be due to widely different causes; some of the causes may be purely genetic, others purely environmental, and still others to different combinations of genetic and environmental factors. These different factors and combinations of factors may differently influence milk secretion at different ages and weights.

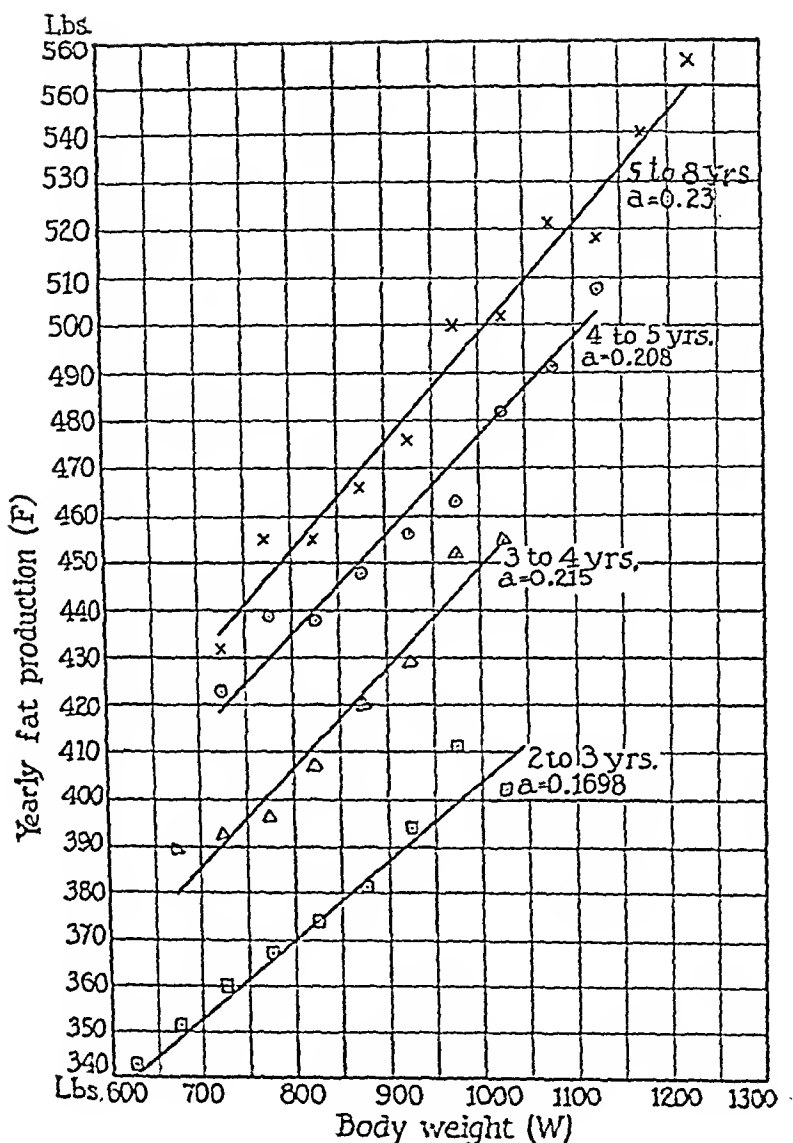


FIG. 3. The increase of milk secretion with increasing body weight at constant age. The smooth lines passing through the observed values were plotted from the equation $F = aW + b$ in which F is the yearly milk fat production for the body weight (W) at the constant ages indicated on the curves; a is the constant increase of yearly fat production for each added pound of body weight. From the values of a indicated on the curves, an increase of 100 pounds in the weight of the body is accompanied by an increase of about 20 pounds of milk fat production per year.

an increase in body weight of 100 pounds is accompanied by an increased yearly butter fat production of about 20 pounds.⁵ The relation between body weight and milk secretion at constant age is linear as would be expected from the approximately linear relation between body weight and blood volume⁶ which carries the nutrients for milk secretion, and from a consideration of certain work pointing to a direct proportionality between the available nutrients in an organism, and its physiological products.⁷ From a comparison of the curves

TABLE II.

The Increase of Milk Secretion with Increasing Body Weight at Constant Age of Register of Merit Jersey Cattle.

Body weight.	Age of cows.							
	2 to 3 yrs.		3 to 4 yrs.		4 to 5 yrs.		5 to 8 yrs.	
	No. of animals included.	Yearly fat production.	No. of animals included.	Yearly fat production.	No. of animals included.	Yearly fat production.	No. of animals included.	Yearly fat production.
<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>
625—	101	342						
675—	199	351	43	389				
725—	540	360	142	392	38	423	52	432
775—	584	367	255	396	101	439	156	455
825—	1043	374	573	407	315	438	458	455
875—	576	381	508	420	318	448	543	466
925—	496	394	605	429	445	456	866	476
975—	155	411	220	452	262	463	599	500
1025—	97	402	185	455	240	482	680	502
1075—					82	491	224	521
1125—					49	508	154	518
1175—							29	540
1225—							27	556

⁵ It may be pointed out that the discovery that there is an increase of 20 pounds of fat production per year for an increase of 100 pounds of body weight at constant age, answers the widely discussed question among dairymen concerning the relative economy of milk production from heavy and light cows. 20 pounds of butter fat is barely sufficient to cover the cost of maintenance of 100 pounds of body weight per year.

⁶ Donaldson, H. H., *The rat*, Memoir of The Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, 1915, 96.

⁷ Cf. Loeb, J., *J. Gen. Physiol.*, 1919-20, ii, 297.

of Figs. 1 and 3, it appears that increasing body weight with age contributes about 20 per cent to the increased milk flow with age, while the other 80 per cent of increased milk flow with age is due to other factors accompanying increased maturity.

The fact that the course of milk secretion with age is largely independent of body weight is also shown in Table III where the com-

TABLE III.

The Increase of Milk Secretion with Age at Constant Body Weight of Register of Merit Jersey Cattle.

Age.	Weight of cows.															
	750 to 849 lbs.				850 to 949 lbs.				950 to 1049 lbs.				1050 to 1151 lbs.			
	No. of cows included.	Yearly fat production.		No. of cows included.	Yearly fat production.		No. of cows included.	Yearly fat production.		No. of cows included.	Yearly fat production.					
		(1)			(2)			(3)			(4)					
		Observed.	Calculated.		Observed.	Calculated.		Observed.	Calculated.		Observed.	Calculated.				
yr.	lbs.															
2.5	1869	354	346	1289	385	381	259	405	406	23	418	389				
3.5	799	401	401	1241	429	430	422	458	453	44	431	449				
4.5	446	433	434	1019	458	457	513	474	478	114	496	486				
5.5	239	463	454	842	483	472	476	496	491	131	519	507				
6.5	174	452	466	563	475	480	503	503	498	97	524	520				
7.5	130	470	474	443	487	485	499	499	501	91	529	528				
8.5	87	479	478	255	487	487	492	492	503	47	533	533				

(1) Computed from $F = 485(1 - e^{-0.12t})$ in which F is the fat production for the age (t).

(2) Computed from $F = 490(1 - e^{-0.12t})$.

(3) " " $F = 505(1 - e^{-0.12t})$.

(4) " " $F = 540(1 - e^{-0.12t})$.

puted and observed milk secretions at different ages are given for the groups of animals at constant body weight. The equation of a monomolecular reaction which represents growth in body weight and increase in milk secretion with age shown in Fig. 2, also represents fairly satisfactorily the increase in milk secretion with age at constant body weight shown in Table III.

SUMMARY.

It is shown that from 2 years, the age when milk secretion usually begins, to 9 years, the age of maximum body weight, the increase of milk secretion with age follows the course of growth in body weight—both can be accurately represented by the equation of a monomolecular chemical reaction having a velocity constant of approximately the same numerical value. While increase in milk secretion and increase in body weight with age follow the same course, it is shown that increasing body weight contributes only about 20 per cent to increasing milk secretion with age. The fact that milk secretion and body weight follow the same course, even though they are largely independent of each other indicates that increase in body weight is a good measure of growth of the dairy cow; this fact also shows that the increase of milk secretion with age may be used as a measure of growth. The fact that milk secretion, like body weight, follows the course of a chemical reaction, adds further support to the theory that growth is limited by a chemical reaction.

THE RATE OF GROWTH OF THE DAIRY COW.

IV. GROWTH AND SENESCENCE AS MEASURED BY THE RISE AND FALL OF MILK SECRETION WITH AGE.

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(Received for publication, July 17, 1923.)

The increase of milk secretion and body weight in the dairy cow follows exactly the same course from the age when milk secretion usually begins (2 years), to the age when maximum body weight is reached (about 8 years).¹ It is therefore concluded that the increase of milk secretion with age may be used as a measure of growth in the same sense that the increase of body weight with age is used as a measure of growth. After the age of maximum body weight is reached, it remains practically constant and can therefore no longer be used to measure the effect of age on the condition of the body; but milk secretion takes a downward course after passing the age of maximum body weight and steadily declines with age. This steady decline of milk secretion with age suggests the possibility of using milk secretion as a measure of the effect of age on the body after age ceases to have an appreciable effect on body weight—in other words, to use the declining curve of milk secretion as a measure of senescence. In the absence of contradictory evidence, there seems no reason to believe why the course of milk secretion which was found to be a good quantitative measure of growth during the growing phase of life, should not also be a good quantitative measure of senescence during the declining phase of life. Adopting the point of view that the rising and falling curve of milk secretion with age is a representation of developing and declining physical powers with age due to the processes classed under growth and senescence, we have brought together a large amount of data on the change of milk secretion with

¹ Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1923-24, vi, 21.

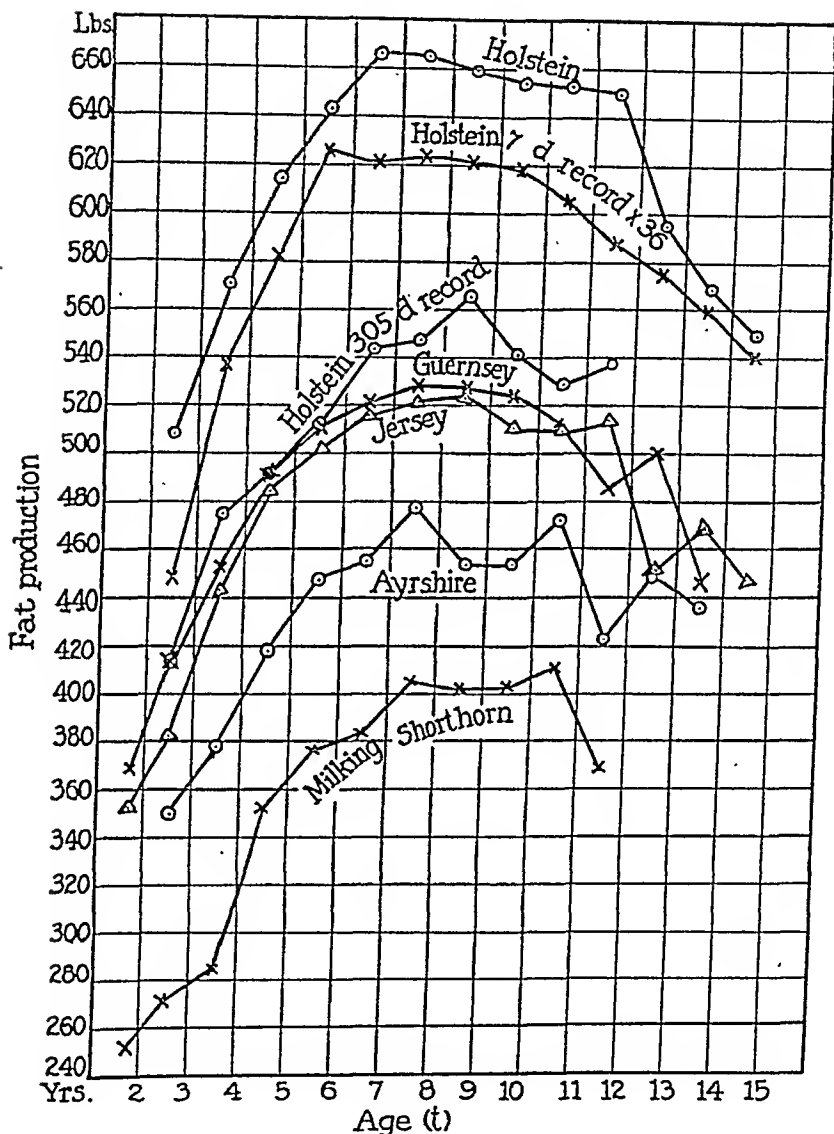


FIG. 1. The course of milk secretion with age for several breeds of dairy cattle plotted from data in Table I. With the exceptions noted on the curves, the curves represent the butter fat production per year of 365 days. For the Guernsey cows, the records represent 365 days or less, if the cow happened to dry off before the end of the year. The curves in this figure represent a total of 150,544 records.

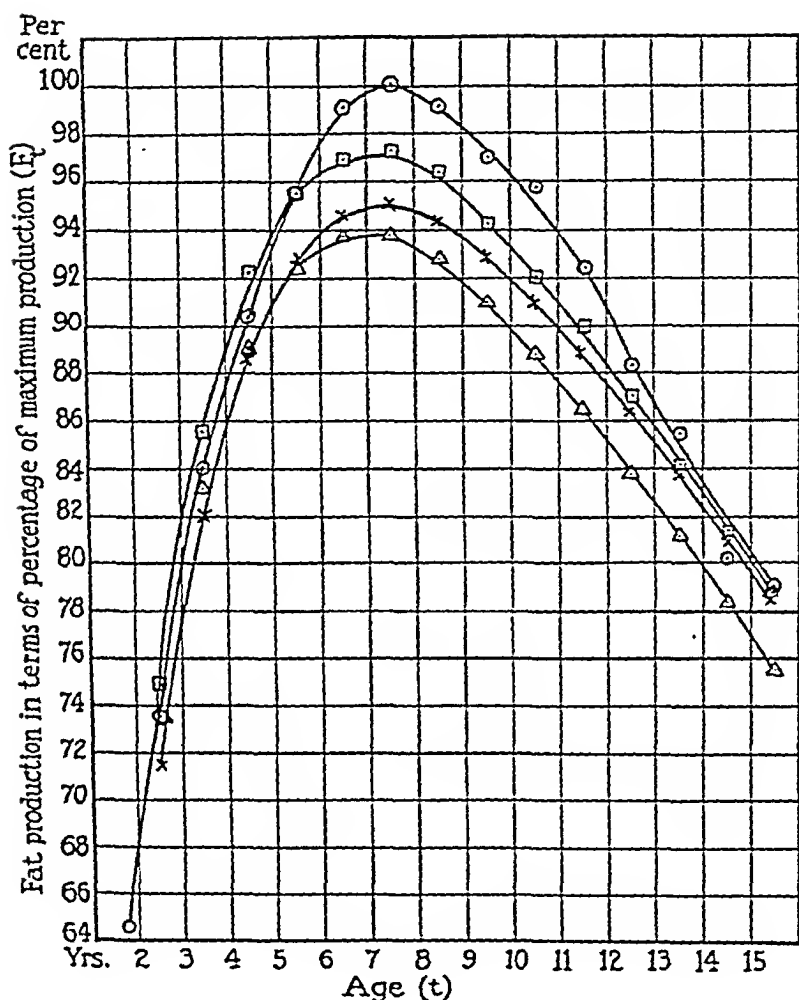


FIG. 2. Comparison between computed and observed values of milk secretion with age for yearly records. The circles represent observed values of the weighted averages of all cows yearly and 305 day records expressed as percentage of maximum production given in table I. The other values were obtained from trial equations as follows: \square represents $F_t = 142.4e^{-0.277t} - 126.15e^{-0.224t}$; $+$ represents $F_t = 142.4e^{-0.277t} - 126.15e^{-0.210t}$; Δ represents $F_t = 142.4e^{-0.277t} - 120e^{-0.210t}$. After the age of 12 years, the observed values are not reliable due to the small number of animals represented. The observed values in this figure are based upon 45,984 yearly and 10 monthly records.

age for the purpose of presenting a continuous quantitative picture of the two phases of life, and, if possible, to formulate a rational theory of the quantitative changes of the physical powers during growth and senescence.

Figs. 1, 2, and 3 plotted from data in Table I show the rise and fall of milk secretion with age from 2 years, the age when milking usually begins, until 14 years. These curves represent, of course, only a portion of the picture of rising and falling physiological activities of

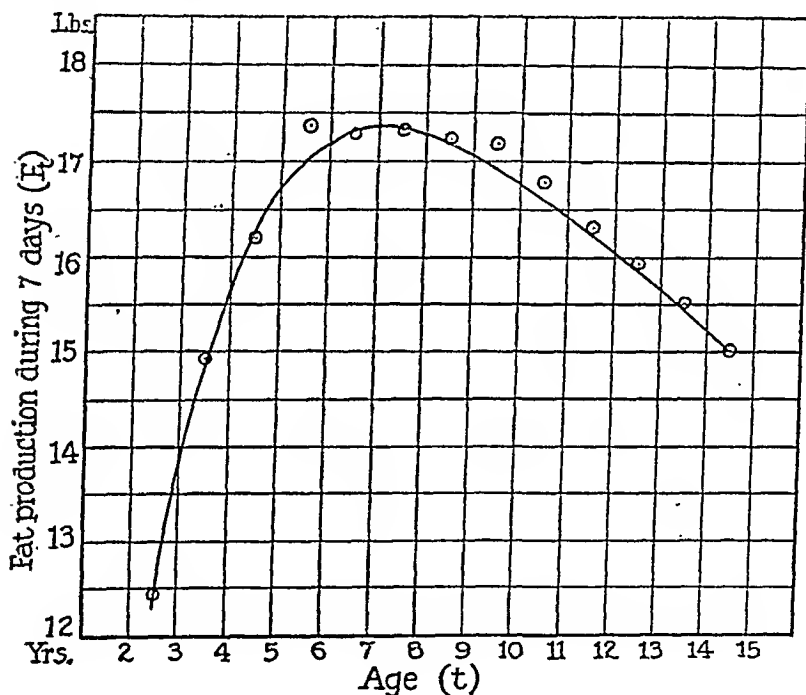


FIG. 3. Comparison between computed and observed values of milk secretion with age for 7 day records given in Table I. The circles represent observed values, the smooth line represents the equation $F = 22.1(e^{-0.026t} - 1.2e^{-0.46t})$. The observed values in this figure are based on a total of 104,560 records. The rate of senescence, it may be noted, is less steep than the rate of growth. The relative slopes of the two sides of the curve at any ages t and t_2 , may be found by substituting the ages for t in the equation $\frac{dF}{dt} = 22.1(-0.026e^{-0.026t} + 1.2 \times 0.46e^{-0.46t})$.

TABLE I.

*Data on the Course of Milk Secretion with Age in the Dairy Cow.**

Data on the Course of Milk Secretion with Age in the Dairy Cow.																								
Age.	Ayrshire cows.			Guernsey cows.			Holstein cows.				Jersey cows.		Milking Shorthorn cows.		Weighted average of all cows.		7 day records, Holstein cows.		7 month records of a group of long-lived cows.					
	365 day records.		305 day records.	365 day records.		305 day records.	Fat 365 days.		Fat 305 days.	Fat per yr.		Fat per yr.	Fat expressed as per cent of maximum production.		Fat 7 days.	Fat per 7 mos.	No. of cows included.	Fat 7 days.	No. of cows included.	No. of cows included.	Fat per 7 mos.			
	No. of cows included.	Fat per yr.		No. of cows included.	Fat 365 days.		No. of cows included.	Fat 305 days.		No. of cows included.	Fat per yr.		No. of cows included.	Fat per yr.								No. of cows included.	Fat expressed as per cent of maximum production.	
yr.																								
1 7	1,710	350		313	368																			
2 5	903	378		5,241	412	2,454	508	1,200	413															
3 5	716	418		2,566	452	1,523	570	762	475															
4 5	515	448		1,977	492	1,238	615	606	493															
5 5	399	455		1,133	511	1,116	644	467	513															
6 5	298	478		897	521	835	666	331	544															
7 5	225	453		572	528	583	665	223	547															
8 5	155	451		369	527	396	659	156	566															
9 5	100	472		261	524	232	654	72	541															
10 5	52	423		123	512	111	652	46	529															
11 5	26	450		76	486	59	650	17	538															
12 5	15	436		32	500	37	595	13	411															
13 5	8	375		21	447	11	569	6	510															
14 5	4	392		5	491	4	550																	
15 5				4	446	4	475																	
Total†	5,156			13,596		8,603		3,899																

* Compiled from the records of Register of Merit Jersey, Record of Merit Shorthorn, Advanced Register Ayrshire, Guernsey, and Holstein cattle.

† Total number of cows included in this table is 150,544 (exclusive of longlived cows).

age for the purpose of presenting a continuous quantitative picture of the two phases of life, and, if possible, to formulate a rational theory of the quantitative changes of the physical powers during growth and senescence.

Figs. 1, 2, and 3 plotted from data in Table I show the rise and fall of milk secretion with age from 2 years, the age when milking usually begins, until 14 years. These curves represent, of course, only a portion of the picture of rising and falling physiological activities of

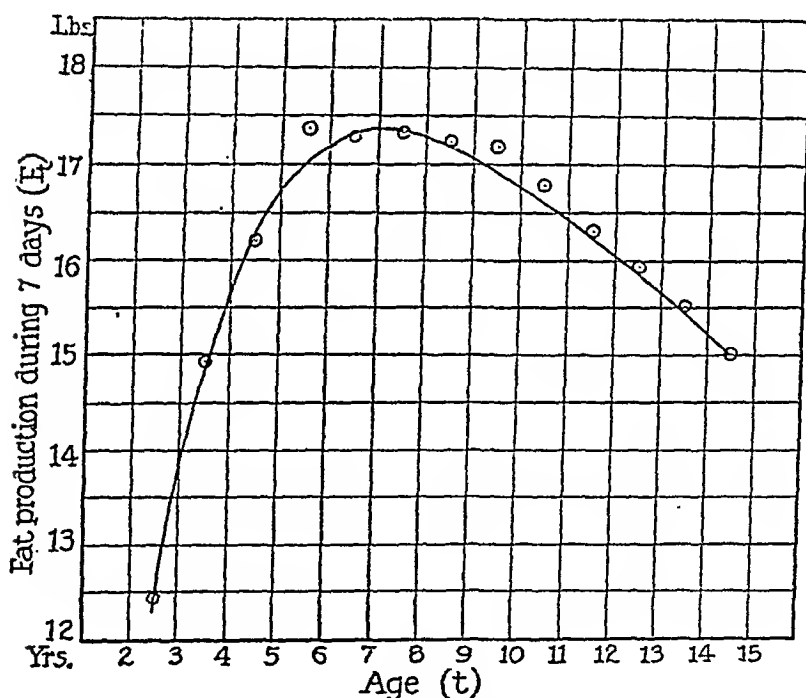


FIG. 3. Comparison between computed and observed values of milk secretion with age for 7 day records given in Table I. The circles represent observed values, the smooth line represents the equation $F = 22.1(e^{-0.026t} - 1.2e^{-0.46t})$. The observed values in this figure are based on a total of 104,560 records. The rate of senescence, it may be noted, is less steep than the rate of growth. The relative slopes of the two sides of the curve at any ages t and t_2 , may be found by substituting the ages for t in the equation $\frac{dF}{dt} = 22.1(-0.026e^{-0.026t} + 1.2 \times 0.46e^{-0.46t})$.

TABLE I.
Data on the Course of Milk Secretion with Age in the Dairy Cow.*

Age.	Ayrshire cows.		Guernsey cows.		Holstein cows.		Jersey cows.		Milking Shorthorn cows.		Weighted average of all cows.	7 day records, Holstein cows.		7 month records of a group of long-lived cows.	
yr.	No. of cows included.	Fat per yr.	No. of cows included.	Fat per yr.	365 day records.	305 day records.	No. of cows included.	Fat 365 days.	No. of cows included.	Fat 365 days.	No. of cows included.	Fat 7 days.	No. of cows included.	No. of cows included.	Fat per 7 mos.
1 7	1,710	350	313	368	2,154	1,200	947	353	15	252	1,275	64.6	33,765	21	24.0
2 5	903	378	5,241	412	1,523	570	4,090	383	306	272	15,001	73.6	22,019	24	28.1
3 5	716	418	2,566	452	1,328	615	2,263	413	167	285	8,184	84.0	16,374	33	33.1
4 5	515	448	1,977	492	1,238	615	1,687	486	125	352	6,349	90.4	11,259	33	35.0
5 5	399	455	1,133	511	1,116	614	1,487	502	75	376	4,823	95.5	8,356	37	37.4
6 5	298	478	897	521	835	666	1,067	516	80	383	3,609	99.1	5,586	32	37.1
7 5	225	453	572	528	583	665	837	521	66	405	2,579	100.0	3,256	40	38.7
8 5	155	451	369	527	396	659	565	524	65	402	1,776	97.0	1,862	39	36.9
9 5	100	472	264	521	232	654	355	510	43	403	1,121	99.1	1,054	39	37.9
10 5	52	423	123	512	111	652	200	509	29	411	609	95.8	548	39	33.8
11 5	26	450	76	486	59	650	108	513	21	369	333	92.4	16.34	32	32.1
12 5	15	436	32	500	37	595	58	453	13	397	179	88.3	285	18	33.3
13 5	8	375	24	447	11	569	31	470	4	399	91	85.5	130	15	34.8
14 5	4	392	5	401	4	550	13	447	3	360	33	80.2	42	15	34.8
15 5			4	446	1	475	8	451	2	353	22	79.1	24	15	34.8
Total†	5,156		13,596		8,603	3,899	13,716		1,014		45,984		104,560		

* Compiled from the records of Register of Merit Jersey, Record of Merit Shorthorn, Advanced Register Ayrshire, Guernsey, and Holstein cattle.

† Total number of cows included in this table is 150,544 (exclusive of long-lived cows).

this animal. The natural duration of life of the cow is said to be about 30 years. Milk records above 15 years are, however, very scarce because in addition to the accidental deaths which leave relatively few animals by the time this age is reached, the animals are also purposely disposed of due to the fact that they become increasingly unprofitable milk producers with increasing age caused by such factors as the increasing difficulty of breeding with age and decaying teeth.

While these curves represent only about half of the whole life curve, they are nevertheless of value because they show the trend of relative rates of growth and of senescence, and the age of the statistical equilibrium between these two processes. Besides, if the growth and senescence curves continue an unchanged course beyond the range of observation, as is probable, then it should be possible to extrapolate the curves by the use of some suitable formula, thereby completing the whole picture.

The trend of the curves of growth and of senescence having been determined on the basis of an extensive amount of data (there is no doubt that the data presented here are by far the largest amount that was ever brought together on the quantitative variation of milk secretion with age, and with the exception of vital statistics on man, they are the largest body of quantitative data on the change of any physiological activity with age), the next step is to formulate a mathematical expression which would not only represent the empirical curve, but what is far more important, an expression which should be rational in the sense that it is derived theoretically as a conclusion from a general law of nature, and which should be capable of explaining the mechanism of the process under investigation. Pearl and his coworkers² found that the expression

$$y = a + cx^2 + d \log x$$

in which y is the milk production and x is the age, may be accurately fitted to the variation of milk secretion with age. However, since

² Pearl, R., and Patterson, S. W., *Maine Agric. Exp. Station, Bull.* 262, 1917. Pearl, R., and Miner, J. R., *J. Agric. Research*, 1919, xvii, 285. Pearl, R., Gowen, J. W., and Miner, J. R., *Ann. Rep. Maine Agric. Exp. Station 1919*, 1919, 89. Gowen, J. W., *Genetics*, 1920, v, 111. Gowen, J. W., *Ann. Rep. Maine Agric. Exp. Station 1920*, 1920, 185.

Pearl and his coworkers do not state that this equation explains the mechanism of this variation of the activity of the mammary gland with age, or that the equation was derived as a conclusion from a general law then this is probably an empirical equation and it does not solve the problem of formulating a rational equation explaining the mechanism of the peculiar course of growth and of senescence.

The following theory is suggested as a basis for formulating a rational equation to represent the rising and falling curve of physiological activities with age and therefore as a basis for a quantitative theory of growth and senescence. It is assumed that growth and senescence go on simultaneously from the beginning to the end of life, but that the ratio between the velocities of these two processes varies in a continuous manner throughout life. At the beginning of life the ratio of growth to senescence is infinitely great, while at the time of natural death, at the extreme old age, the ratio of senescence to growth is infinitely great. At the age of maximum, or prime physical development the two processes just balance each other. The view is also adopted that growth and senescence are physico-chemical processes governed by the laws of mass action—a view due to Loeb and his coworkers.³ If growth and senescence are physico-chemical processes, then the course of each, growth and senescence, should follow the course of some chemical reaction, since it is a general principle of chemistry that in a system of chemical reactions which are interdependent, the slowest reaction determines the rate of the resultant process,⁴ and therefore growth and senescence while complicated processes should theoretically follow the course of chemical reactions of a simple order; and if growth and senescence go on simultaneously, but with a continuously changing ratio of velocities, then it should be possible to express the course of the whole curve of growth and senescence by the mathematical expression which represents the course of simultaneous consecutive chemical reactions. According to this theory, life may be represented symbolically by the expression $X \rightarrow Y \rightarrow Z$ in which $X \rightarrow Y$ is the process of

³ Cf. (inter alia) Loeb, J., *Biochem. Z.*, 1906, i, 183. Loeb, J., and Lewis, W. H., *Am. J. Physiol.*, 1901-02, vi, 305. Loeb, J., and Northrop, J. H., *Proc. Nat. Acad. Sc.*, 1916, ii, 456; 1917, iii, 382. Loeb, J., *Scienc. Monthly*, 1919, ix, 578.

⁴ Cf. Walker, J., *Proc. Roy. Soc. Edinburgh*, 1897-98, xvii, 22.

growth which follows the course of a chemical reaction, or reactions, as we have indeed found in studying the growth of the dairy cow,⁵ the animal under consideration, and as was found to be the case for the growth of many other organisms;⁶ $Y \rightarrow Z$, the process of senescence which it is assumed also follows the course of, and is limited by some chemical reaction. The whole process $X \rightarrow Z$ can therefore probably be represented by the equation of some consecutive reactions; for example,

$$M_t = A (ae^{-k_1 t} - be^{-k_2 t}) \quad (1)$$

in which k_1 and k_2 are the velocity constants respectively of senescence and growth, M_t , the milk production (or other index of physiological activity with age) at the age, t , and e , the base of natural logarithms.⁷

This equation (1) was in fact found to represent the course of milk secretion with age quite satisfactorily as may be seen in Figs. 2 and 3 where the observed values are fairly close to the values computed from this equation. This equation cannot be rigidly tested, first, because the theory of consecutive reactions is incomplete; second, because since growth and senescence are simultaneous, it is not possible to determine separately the values of the velocity constants k_1 and k_2 . The satisfactory fit of the trial equation to the data is, however, extremely suggestive. The oxidation of phosphorous acid by potassium persulfate in the presence of hydriodic acid, is a classical example of consecutive simultaneous reactions. The numerical values of the velocity constants for this reaction were determined separately for the constituent reactions, the reduction of the persulfate by hydriodic acid, and the oxidation of phosphorous acid by iodine. The comparison between the theoretical and observed

⁵ Brody, S., and Ragsdale, A. C., *J. Gen. Physiol.*, 1920-21, iii, 623. Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1923-24, v, 445.

⁶ Cf. (*inter alia*) Robertson, T. B., *Principles of biochemistry*, for students of medicine, agriculture, and related sciences, Philadelphia and New York, 1920.

⁷ For derivation and application of this equation to certain life processes in plants cf. Osterhout, W. J. V., *Injury, recovery, and death, in relation to conductivity and permeability*, Monograph on Experimental Biology, Philadelphia and London, 1922.

values of this reaction *in vitro*⁸ and the values obtained from trial equations and observed values in Fig. 2 and especially in Fig. 3 in the variation of milk secretion with age is not unfavorable to the latter.

The fit of the computed to the observed values of milk secretion in Fig. 1 is especially satisfactory considering some of the defects of the observed data. The most serious defect is that the population rapidly decreases with age due to the natural and purposeful elimination of the less fit animals, either because of defective physical vigor causing death at an early age, or because of unsatisfactory milk production. As a result of this elimination, and the elimination due to the minimum entrance requirements for Advanced Registry, only the better animals survive at the more advanced ages, amounting to a comparison between relatively good animals at the more advanced ages with relatively mediocre animals at the earlier ages. This would tend to a relatively higher production at the more advanced ages which may explain the fact shown in Fig. 2 that at later ages the observed production is above the computed production. The increasing difficulty of breeding animals with increasing age resulting in a longer farrow period may likewise increase the relative milk production at the later ages, since it has been shown⁹ that pregnancy appreciably decreases milk production. The 7 day data shown in Fig. 3 are superior to the yearly data of Fig. 2, first, because the effect of delayed breeding due to age is absent since the 7 day records are always made before breeding; second, because the 7 day records are made when the animals are at their best, shortly after calving; and third, and most important, since the period of test is only 7 days the animals can be kept for this brief period under the very best conditions, eliminating the many unfavorable factors which come up during the course of a whole year. These uniform and favorable conditions under which the 7 day records are made therefore represent more nearly the genetic capacity of the animals than the yearly records in which environmental factors are less under control. These

⁸ Federlin, W., *Z. physikal. Chem.*, 1902, xli, 565. Lewis, W. C. McC., *A system of physical chemistry*, London and New York, 1920, 3rd edition, 403, 4.

⁹ Brody, S., Ragsdale, A. C., and Turner, C. W., *J. Gen. Physiol.*, 1922-23, v, 777.

facts and the fact that the 7 day averages are based on a very much larger number of animals than the yearly averages, may explain the better agreement between observed and computed values for the 7 day records in Fig. 3 than the yearly records in Fig. 2.

SUMMARY.

Data are presented on the effect of age on milk secretion in the dairy cow. From the age when milk secretion usually begins (2 years) to the age when maximum body weight is reached (about 8 years) increase of milk secretion and increase of body weight with age follow the same exponential course; which is the course of a monomolecular reaction of chemistry. After this age, unlike body weight which remains practically constant, milk secretion declines exponentially, that is, the course of decline follows the course of decline of a monomolecular reaction. The whole course of milk secretion with age was therefore found to follow approximately the course of two simultaneous, consecutive, monomolecular reactions. This is taken to mean that growth and senescence go on simultaneously from the beginning to the end of life, and that each follows an exponential law with age; and therefore perhaps that the course of the two processes are limited by two consecutive chemical reactions.

THE RATE OF SENESCENCE OF THE DOMESTIC FOWL AS MEASURED BY THE DECLINE IN EGG PRODUCTION WITH AGE.

By SAMUEL BRODY, EARL W. HENDERSON, AND H. L. KEMPSTER.

(From the Agricultural Experiment Station, University of Missouri, Columbia.)

(Received for publication, July 17, 1923.)

It is well known among poultrymen that egg production in the domestic fowl declines with age. Indeed, it is the general opinion that the decline is so rapid that after the age of 2, or at most 3 years, a hen is no longer a profitable egg producer, and should therefore be disposed of. The decline in egg production with age may be due to the gradual exhaustion of the oocytes, or to the decline in vigor of the organs or tissues which form the limiting factors in egg production, or to both. In either case, whether it be exhaustion of a limited substance on which the process depends, or whether it be the wearing out of a limiting organ, the process may be classed as senescence, and the course of egg production with age may be used as an index of the course of senescence of the limiting tissues or organs. If this reasoning is correct, then data on the course of decline of egg production with age should be suitable material for testing by one method the theory that senescence is a physicochemical process.

If senescence is a physicochemical process, then the course of senescence should follow the course of some chemical reaction, since it is a general principle of chemistry that in a system of chemical reactions which are interdependent, the slowest reaction determines the rate of the resulting process¹ and therefore senescence, while a complicated process, should roughly follow the course of a chemical reaction of a simple order (the slower the limiting reaction as compared to the other reactions in the system, the closer the agreement between the calculated and observed values). The data on egg production presented in

¹ Walker, J., *Proc. Roy. Soc. Edinburgh*, 1897-98, xxii, 22.

Table I and Fig. 1 were analyzed from this standpoint and they were found to follow the course of the equation

$$E_t = Ae^{-kt} \quad (1)$$

in which E_t is the egg production during any age, t , e is the base of natural logarithms, and A and K are constants. This equation indicates that each year's egg production is, regardless of age, the same per cent of the preceding year's production. Thus in Table I, if any value is

TABLE I.

The Decline in Egg Production with Age in the Domestic Fowl.

Age.	No. of birds included.	Egg production per year. (Nov. 1 to Nov. 1.)	
		Observed.*	Calculated.†
<i>yrs.</i>			
1	222	158	158.5
2	221	140	140.1
3	222	124	123.9
4	222	110	109.6
5	193	95	96.9
6	28	89	85.7
7	27	71	75.7
8	6	63	67.0

* The observed values are averages of two breeds and eight different groups of birds kept under different conditions of management. To save space in this general publication, the individual records, and their significance, are omitted, but they will probably be presented in a poultry journal.

† Calculated from the equation $y = 179.2e^{-0.123t}$ in which y is the yearly egg production at the age, t . The egg production during any year is 88 per cent of the preceding year's production.

divided by the preceding value, the quotient is always .88, that is each year's production is 88 per cent of the preceding year's production. In other words, the percentage decline of egg production with age is constant, or exponential, and the course of senescence of the limiting tissues or organs involved in egg production, may therefore be said to follow an exponential law.

This exponential law represented by equation (1) is the same as the law of monomolecular change in chemistry, thereby substantiating

the suggestion that "the natural duration of life would be in reality the time required to complete a chemical reaction or a series of chemical reactions resulting in the production of toxic compounds sufficient to kill or resulting in the destruction of necessary compounds."²

Equation (1) may also be used to throw some light concerning the particular factor that limits egg production. The total number of

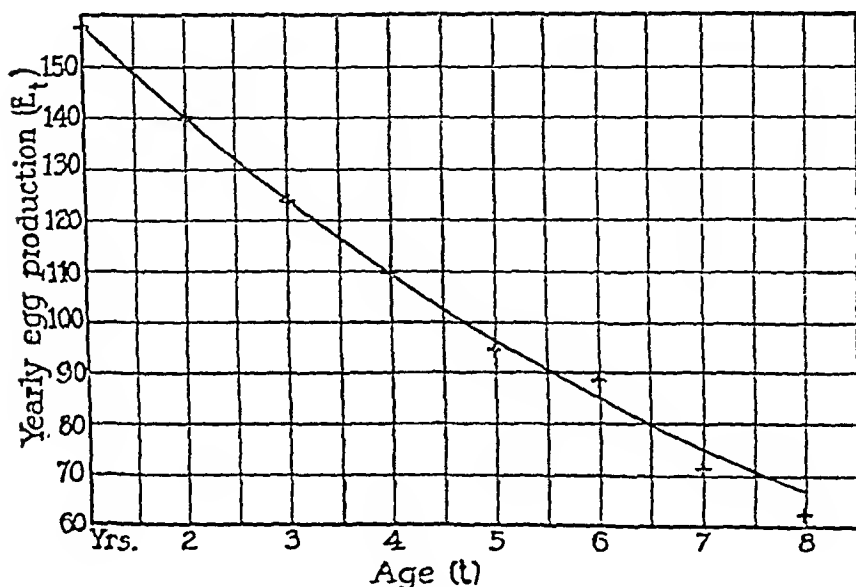


FIG. 1. The decline in egg production with age in the domestic fowl plotted from the averages in Table I. The smooth line represents the computed values; the crosses represent the observed values. The observed values during the 7th and 8th years are not reliable due to the small number of individuals represented in the averages.

eggs the average fowl in Table I would lay if she lived long enough to allow the senescence reaction represented by equation (1) to go to completion would be the total number of eggs laid from the time laying begins ($t = \frac{1}{2}$ year) to the time the senescence reaction is completed ($t = \text{infinity}$). This theoretical number of eggs can be found by

²Loeb, J., *Scient. Monthly*, 1919, ix, 578.

integrating equation (1) between the limits of $\frac{1}{2}$ year and infinity as follows:

$$E = \int_{\frac{1}{2}}^{\infty} A e^{-kt} dt = \frac{a}{-k} \left[-e^{-kt} \right]_{\frac{1}{2}}^{\infty} = \frac{179.2}{0.123} e^{-\frac{0.123}{2}} = 1369$$

Now this total number of eggs, 1369, that the average hen in Table I can theoretically produce falls considerably below the number of oocytes found in the hen. Thus M. R. Curtis³ found an average (in thirteen hens) of 1814 oocytes visible to the naked eye. There must be many more not so visible.⁴ This rather low value obtained from the equation as compared to the actual count seems to indicate that it is not the number of oocytes in the hen that limits the course of egg production, but rather the decline in vigor of some limiting organ or organs.

SUMMARY.

Data are presented showing that the course of decline of egg production with age in the domestic fowl from the time laying begins up to and including 8 years follows an exponential law, that is, each year's egg production is a constant percentage of the preceding year's production (88 per cent in the group of fowl studied). Since the exponential law is the same as the law of monomolecular change in chemistry, and since the course of egg production with age may be taken as an index of the course of senescence of organs, or tissues limiting egg production, it is suggested that this exponential law of egg production substantiates the idea that senescence is a physicochemical process the course of which is limited by a chemical reaction. It is shown that the exhaustion of the oocytes is not likely to be the factor limiting the course of egg production.

There is a good deal of published data on egg production with age up to 4 years. We are not, however, acquainted with statistically significant data after this age. This scarcity of data after 4 years is due to the difficulty of keeping records of egg production after 4 years of birds whose "normal average expectation of life at birth" is not substantially

³ Curtis, M. R., Quoted by Pearl in *Maine Agric. Exp. Station, Bull. 205*, 1912.

⁴ Pearl, R., *Maine Agric. Exp. Station, Bull. 205*, 1912.

more than 2 years."⁵ The relatively large amount of data up to 8 years presented in this communication was made possible by combining data obtained at other places with the data obtained at this station. We take much pleasure in expressing our indebtedness to J. H. Bardsley, of the New Mexico Station, J. E. Daugherty, of the California Station, B. F. Kaupp, of the North Carolina Station, J. H. Martin, of the Kentucky Station, H. G. May, of the Rhode Island Station, and L. H. Schwartz, of the Purdue Station, for their cooperation in obtaining the data summarized in Table I.

We have also included in our averages the data given by E. D. Ball, G. Turpin, and Byron Alder in the Utah Agricultural Experiment Station Bulletins.⁶

⁵ Pearl, R., *The biology of death*, Monograph on Experimental Biology, Philadelphia and London, 1922, 63.

⁶ Ball, E. D., Turpin, G., and Alder, B., *Utah Agric. Exp. Station, Bull.* 135, 1914. Ball, E. D., Alder, B., and Egbert, A. D., *Utah Agric. Exp. Station, Bull.* 148, 1916.

FURTHER EXPERIMENTS ON THE ABSORPTION OF IONS BY PLANTS, INCLUDING OBSERVATIONS ON THE EFFECT OF LIGHT.

By D. R. HOAGLAND AND A. R. DAVIS.

(From the Division of Plant Nutrition, College of Agriculture, University of California, Berkeley.)

(Received for publication, July 16, 1923.)

Several investigations dealing with the penetration of ions into the cell sap of *Nitella*¹ have been reported recently by Osterhout,² Irwin,³ Brooks,⁴ and by the writers.⁵ It has been proved by the experiments already described that most of the inorganic elements of the cell sap are present in dissociated form, and that these plant cells are able to cause the movement of ions from a solution of low concentration into one of higher concentration. As suggested in a previous article, this condition requires that energy relations be taken into account, in other words, the plant apparently must do work in absorbing ions from dilute solutions. If this is the case, it is reasonable to suppose that for autotrophic plants, light, either directly or indirectly, is necessary to the processes of absorption, since light furnishes the ultimate source of energy to the plant. We have, therefore, made observations on the removal of certain ions from dilute solutions by cells of *Nitella clavata* kept under varying conditions of illumination. In the preliminary experiments, qualitative tests for the removal of chlorine from solution were made by comparisons of the turbidity developed when the chloride was precipitated by the

¹ As noted in a previous paper, the observations of Wodehouse on *Valonia* in 1917 first directed the attention of the writers to the advantage of supplementing their absorption studies on higher plants by the use of individual plant cells. At that time, Dr. Nathaniel Gardner kindly suggested the possibility of *Nitella*.

² Osterhout, W. J. V., *J. Gen. Physiol.*, 1921-22, iv, 275.

³ Irwin, M., *J. Gen. Physiol.*, 1922-23, v, 223.

⁴ Brooks, M. M., *J. Gen. Physiol.*, 1921-22, iv, 347.

⁵ Hoagland, D. R., and Davis, A. R., *J. Gen. Physiol.*, 1922-23, v, 629.

esters (30.08 Gm.), and the mixed derivatives were fractionated at 5 mm. pressure in a Todd pressure fractionation assembly (see Table V).

Fraction one was hydrolyzed with alcoholic KOH and the acid thus liberated was isolated and purified. Its molecular weight, determined through the neutralization equivalent (229.9), corresponded to that of myristic acid (mol. wt. 228.36). To further confirm its identity, the acid was converted to the hydroxamate which melted at 97.5–98.5°. Myristohydroxamic acid melts at 98–98.5°.

Fraction two, on hydrolysis with alcoholic KOH, yielded an acid whose neutralization equivalent was 254.7, which compares favorably with the molecular weight of palmitic acid (256.42). The hydroxamate of the acid melted at 102–103°. Palmitohydroxamic acid melts at 102.5°.

Fraction three and the residue were combined and hydrolyzed as above. The acid recovered had the molecular weight 280.2, which compares with that of stearic acid (M. W. 284.47). The hydroxamate, melting at 105–106°, was concluded to be stearohydroxamic acid, m. p. 106–107°.

Unsaturated Fatty Acids—The separation of the unsaturated fatty acids (1.1 Gm.) was accomplished by the standard bromination procedure. The absolute ether solution of the mixed bromides yielded no crystalline matter, indicating the absence of linolenic acid. This is confirmed by the thioevanogen number (Table III).

The petroleum ether solution yielded 0.66 Gm. of tetrabromolinolenic acid, m. p. 115°, corresponding to 28.0% of the unsaturated acid fraction.

Dibromo oleic acid recovered from the petroleum ether filtrate was debrominated with zinc dust in the usual manner, yielding 0.85 Gm. of oleic acid, corresponding to 72.0% of the unsaturated acid fraction. Its iodine value was 85.48 and its molecular weight was 280.

TABLE V — COMPOSITION OF THE MIXED FATTY ACIDS

Acids	Weight %	Molar %
Myristic	36.0	32.33
Palmitic	33.1	33.37
Stearic	4.5	5.03
Oleic	20.1	22.32
Linoleic	6.3	6.95

Component Glycerides of the Whole Fat—The saturated glycerides were isolated from the whole fat by the method of Hilditch and Lea (7). Following permanganate oxidation in dry acetone, 17.64 Gm. of neutral fat yielded 9.49 Gm. of saturated glyceride, corresponding to 48.13% of the neutral fat. Its chemical constants were: saponification value 217.0, iodine value 0.0, acid value 0.4.

Based upon the selectivity of KHCO_3 and K_2CO_3 solutions, Hilditch (8) described the resolution of the oxidation products produced in the determination of saturated glycerides. Mono- and diunsaturated glycerides were thus isolated from the reaction mixture, however, no triunsaturated glyceride was found. Kartha and Menon (9) used the saponification value of these cleavage products to calculate the mono- and diunsaturated glyceride content of a fat. The

yield of mono- and diunsaturated glyceride was 4.5333 Gm., saponification value 316.2. The projection of these data in the manner described by Kartha and Menon gave rise to the percentage composition of the glycerides of poison ivy fat (see Table VI).

TABLE VI — COMPONENT GLYCERIDES OF THE POISON IVY FRUIT FAT

Glycerides	Weight %
Trisaturated	48.13
Disaturated monounsaturated	22.01
Monosaturated diunsaturated	29.86

Unsaponifiable Matter.—The ethereal solution of the unsaponifiable matter was alternately washed with dilute NaOH solution and water. After drying over anhydrous Na_2SO_4 , the solvent was recovered under reduced pressure, leaving a residue which weighed 0.776 Gm., corresponding to 0.776% of the whole fat.

A portion of this material (225.1 mg.) dissolved in a minimum of petroleum ether was placed on a column (2 x 30 cm.) of adsorption alumina (Fisher Scientific Co., 80–200 mesh). The column was successively eluted with 150 ml. each of petroleum ether, carbon tetrachloride, benzene, and chloroform containing 10% ethanol. The extracts were freed of their solvents, dried in a vacuum, and weighed.

The petroleum ether extract (30.4 mg.) was a colorless liquid which congealed when cooled slightly and which was soluble in concentrated H_2SO_4 . It decolorized bromine water and had an iodine value of 20.44, $n_D^{20} = 1.4700$. Presumably it was a hydrocarbon (10, 11) but it was not identified.

The carbon tetrachloride eluate yielded 11.2 mg. of brown, waxy material. Although waves have been reported in this fraction (10), the amount was too small for identification.

The benzene eluate contained pigments as could be judged from its deep yellow color. The residue (13.3 mg.) was rechromatographed in the hope of isolating carotenes (5). Spectrophotometric analysis failed to give a rational absorption pattern, whereas a chloroform solution of standard β -carotene gave an absorption maximum at 462 m μ .

The chloroform ethanol eluate yielded 161.6 mg. of residue which responded strongly to the Liebermann-Burchard test. An alcoholic solution of it was purified by filtration through charcoal. The clear filtrate deposited lustrous white platelets melting at 136.5–137°. Purification by chromatography and fractional recrystallization gave only one compound melting at 137°. This melting point corresponds with that of β -sitosterol. The identity of the compound was confirmed by the preparation of its acetate (m. p. 125°) and its benzoate (m. p. 147°), both of which compare favorably with the corresponding derivatives of β -sitosterol.

Percarp and Seed Fat—It was previously stated that the whole fat actually consisted of two portions, namely that from the pericarp and that from the seeds. The former is a soft, grayish-white mass which completely envelops the dark colored, stony seed, whereas the latter is an oil.

Working in batches of 200 Gm., a large quantity of clean, whole poison ivy fruits (devoid of pericarp)

was alternately washed with petroleum ether and rubbed against a No 10 sieve to remove all of the pericarpal fat. The solid fragments together with the residue from recovery of the solvent corresponded to 24.2% of the weight of the whole fruits.

The seeds were dried and then lightly crushed in a drug mill and extracted for ten hours with petroleum ether. The marc was reground and re-extracted. Recovery of the solvent from the combined extracts yielded a clear, golden-yellow oil. The oil content of the seeds was 9.0%, corresponding to 5.5% of the whole fruit.

Both the pericarpal fat and the seed oil were examined in the usual manner, and the results are given in Tables VII and VIII.

TABLE VII —FAT FROM THE PERICARP PORTION OF THE POISON IVY FRUIT

Physical and Chemical Constants	
Refractive index at 60°	1.4470
Specific gravity, 99°/25°	0.8512
Acid value (mg KOH/1 Gm of fat)	2.73
Saponification value	214.10
Iodine value	14.76
Hehner value	95.23
Unsaponifiable matter	0.88

The total fatty acids of the epicarp fat consisted of 88.0% saturated acids and 12.0% unsaturated acids.

TABLE VIII —OIL FROM THE SEEDS OF THE POISON IVY FRUIT

Physical and Chemical Constants	
Refractive index at 25°	1.4690
Specific gravity, 25°/25°	0.8762
Acid value (mg KOH/1 Gm of the oil)	2.2
Saponification value	177.8
Iodine value	125.6
Hehner value	90.53
Unsaponifiable matter	2.04

The total fatty acids of the seed oil consisted of 6.4% saturated acids and 93.5% unsaturated acids.

SUMMARY

Since an extensive investigation on poison ivy plant sap has already been made, the chemical analysis of the lipids of the fruits of poison ivy (*Rhus radicans radicans*) was undertaken to obtain a clearer picture of this notorious plant.

The poison ivy fruit fat obtained by solvent extraction was found to be 29.75 per cent of the whole fruit and was devoid of sulfatides and amino lipids. It is a grayish-white solid soft mass

possessing a mild, bland odor and it does not give any specific color test.

The fatty acids were separated by lead salt-ether method and the individual saturated acids were obtained by fractional distillation of the methyl esters under reduced pressure. The individual unsaturated acids were separated by bromination. The mixed fatty acids were found to contain 36.0 per cent myristic acid, 33.1 per cent palmitic acid, 4.5 per cent stearic acid, 20.1 per cent oleic, and 6.3 per cent linoleic acid.

The glyceride composition of the fat was determined by the acetone-potassium permanganate oxidation method. The fully saturated glycerides were found to be 48.13 per cent, disaturated-monounsaturated 22.01 per cent, and monosaturated-diunsaturated 29.86 per cent.

The unsaponifiable matter which comprised about 0.9 per cent of the fat consisted of hydrocarbon, waxy material, pigments, and a sterol. The sterol was identified as β -sitosterol. Pigment and waxy material were not investigated for lack of sufficient material. Hydrocarbon appeared to be unsaturated, having iodine value 20.44 and refractive index 1.470 at 40°.

Pericarp fat was separated manually and was found to represent 24.2 per cent of the fruit. The mixed fatty acids of this fat contained 88 per cent solid acids and 12 per cent unsaturated fatty acids. Solid acids were individually separated by fractional distillation and were found to consist of myristic and palmitic acids as major components.

The seed oil represented 5.5 per cent of the weight of the fruit. The unsaturated acids in this oil amounted to 93.53 per cent of the total fatty acids.

REFERENCES

- (1) Kunttel, J., *Am J Pharm*, 30, 542(1858)
- (2) Maisch, J., *ibid*, 38, 4(1866)
- (3) Acree, S., and Syme, W., *J Biol Chem*, 2, 547(1906)
- (4) Stevens, A., and Warren, L., *Am J Pharm*, 79, 522(1907)
- (5) "Official Methods of Analysis of the Association of Official Agricultural Chemists," A O A C, Washington 4, D C, 7th ed., 1950, p. 427
- (6) Bertram, S., *Untersuch Lebensm*, 55, 179(1928)
- (7) Hilditch, T., and Lea, C., *J. Chem. Soc.*, 1927, 3106
- (8) Deuel, H., "The Lipids, Chemistry," Volume I, Interscience Publishers, Inc., New York, 1951, p. 157
- (9) Kartha, A., and Menon, K., *Proc Ind Acad Sci*, 17A, 114(1943)
- (10) DeCastro, N., and Jannke, P., *THIS JOURNAL*, 44, 281(1955)
- (11) Bolton, E., and Revis, C., "Oils, Fats and Fatty Foods," J A Churchill, London, Third Edition, 1950, p. 105

A Study of Moisture Vapor Transmission through Closures^{*}

By SEYMOUR M. BLAUG, EUGENE HICKMAN, and JOHN L. LACH

Moisture vapor transmission through closures is an important consideration when packaging pharmaceutical products. It is particularly important when packaging hygroscopic materials or materials that decompose in the presence of trace amounts of moisture. The cap as well as the cap liner must be considered in choosing a proper closure. The barrier efficiency of sixteen liners in metal caps, phenolic, melamine, and mineral filled plastic caps was studied. Of the liners investigated, polyethylene, aluminum foil, plastic-wax saturated pulpboard, and red rubber liners were found to be the most effective moisture vapor barriers, particularly when used in metal closures.

THE SELECTION OF THE BEST possible closure for a specific product is of great importance in the drug and chemical industry. Some of the factors to be considered are the type of product to be packaged, its hygroscopicity, and product reaction with the cap and/or the cap liner. Although the liner may be effective and stable, the material of the cap may react with the product being packaged. This is particularly true of closures for liquid preparations.

A crucial problem for the packaging department is the selection of the best possible closure for dry solid products that decompose in the presence of traces of moisture or are hygroscopic. Ascorbic acid tablets, for example, develop brown spots in a humid atmosphere due to the increased rate of oxidation of the ascorbic acid. The color and stability of many tablet coatings is affected adversely by the presence of moisture.

Metal caps are generally preferred to plastic caps as closures for dry products. High humidity can cause the expansion of plastic caps, particularly those made from a phenolic resin. The expansion of the cap produces "backoff" or a slow unscrewing of the cap (1). In a communication from Eli Lilly and Company it was stated that the company's standard closure for dry products is enameled tin plate, while the standard liner is a waxed paper applied to a pulp backing. Where extremely hygroscopic dry products are involved, the preferred liner is waxed aluminum foil over pulp.

It was the purpose of this study to compare the barrier efficiency of various liners in metal and plastic screw caps against moisture vapor transmission. Sixteen liners were studied in metal caps, phenolic, melamine, and mineral filled plastic caps.

EXPERIMENTAL

Materials and Apparatus.—Anhydrous calcium chloride, 11-dram emerald green glass vials, constant temperature humidity cabinet, semi micro Gramatic balance, Owens-Illinois torque tester, 28-mm metal and plastic screw caps (Tables I and II).

Procedure.—Ten grams of anhydrous calcium chloride was placed into 11-dram vials. The screw caps under investigation were placed on each vial using the Owens-Illinois torque tester. Each cap was applied using 10 inch pounds torque. This gives removal torque values varying between 6 and 7-inch pounds.

The sealed vials were accurately weighed and placed upright in a constant temperature humidity cabinet adjusted to $38^{\circ} \pm 1^{\circ}$ and a relative humidity of $90\% \pm 5\%$. The vials were removed from the humidity chamber at weekly intervals and allowed to equilibrate at room temperature for fifteen minutes before weighing. Immediately after weighing they were returned to the humidity chamber. Three vials were used for each test. Empty vials with caps were used as controls. It was found that the controls gained $9 \text{ mg} \pm 1 \text{ mg}$ after one

TABLE I—METAL CLOSURES^a STUDIED

Number	Liner
1	0 003 in polyethylene extruded on paper
2	0 0035 in aluminum foil laminated to Kraft paper—foil side coated with a pigmented vinyl coating
3	Red rubber
4	Two sides coated paper bonded to wax saturated paper board
5	0 002-in aluminum foil on paper board
6	Pulp and oil waxed
7	Pulp and vinyl
8	Plastic-wax saturated white pulpboard with coating 0 008-0 012 in of a special plastic wax on one surface
9	Pulp and Suran film waxed
10	0 040 in solid polyethylene
11	Pulpboard
12	0 035 in pulp and Kraft paper backed with 0 001 in Mylar polyester film

^a Closures 1-9 were supplied by Crown Cork and Seal Company; Closures 10-12 were supplied by Armstrong Cork Company.

^{*} Received May 3, 1957, from the College of Pharmacy, State University of Iowa, Iowa City.

This research project was conducted under contract from the Armed Services Medical Procurement Agency, Brooklyn, N. Y.

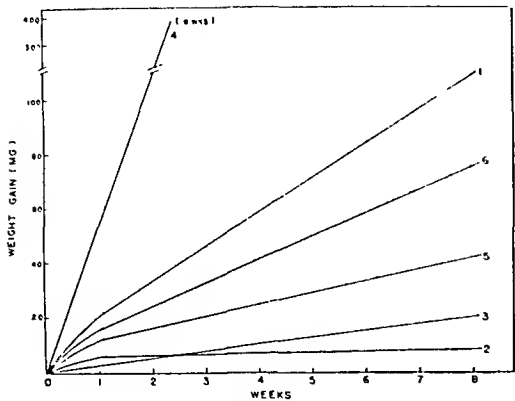


Fig. 1.—Moisture vapor transmission plots-metal closures.

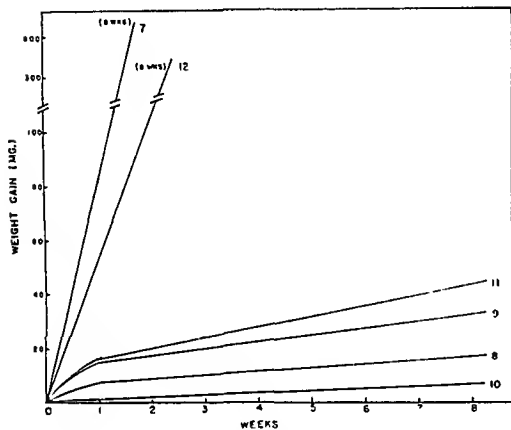


Fig. 2.—Moisture vapor transmission plots-metal closures.

TABLE II.—PLASTIC CLOSURES^a STUDIED

Number	Type Plastic	Liner
13	Melamine	0.040-in. solid polyethylene
14	Phenolic	0.040-in. solid polyethylene
15	Mineral filled	0.040-in. solid polyethylene
16	Melamine	Pulpboard
17	Phenolic	Pulpboard
18	Mineral filled	Pulpboard
19	Phenolic	Polyethylene cone
20	Melamine	Mylar film laminate on pulpboard
21	Phenolic	Mylar film laminate on pulpboard
22	Mineral filled	Mylar film laminate on pulpboard
23	Phenolic	0.040-in. solid polyethylene
24	Phenolic	Aluminum foil
25	Phenolic	Pulp and vinylite waxed
26	Phenolic	Pulp and vinylite lubricant film

^a Closures 13-18 and 20-22 were supplied by Armstrong Cork Co. Closure 19 was supplied by the Poly-Seal Corporation. Closures 23-26 were supplied by Owens-Illinois.

week and remained at this value for the eight-week period of the study. The weight gain in mg. each week is the average gain of the three vials used for each test. Results are shown in Figs. 1-4.

On completion of the study, all caps were removed with the torque tester. Removal torque for metal caps was 6-7 inch-pounds. For plastic caps, the removal torque varied depending on the type of plastic used. Phenolic resin plastic caps gave the lowest removal torques of 4-5 inch-pounds. Melamine and mineral filled plastic caps gave removal torques of 5-7 inch-pounds. The Poly-seal cap gave a removal torque of 6-7 inch-pounds.

DISCUSSION

Results of this study indicate that in general metal closures are more satisfactory than plastic closures as barriers to moisture vapor. The liner greatly affects moisture vapor transmission as does the type of plastic used in the closure. A 0.040-in. solid polyethylene liner in a metal closure is a more satisfactory barrier to moisture vapor than a 0.040-in. solid polyethylene liner in any of the three types of plastic closures used in this study, Figs. 2 and 3. Of the three types

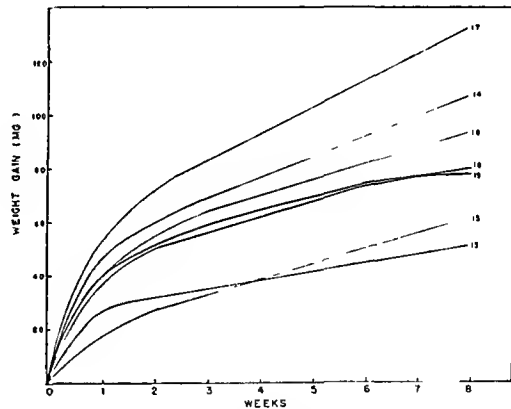


Fig. 3.—Moisture vapor transmission plots-plastic closures.

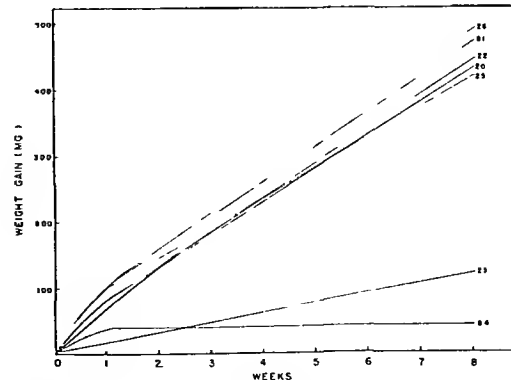


Fig. 4.—Moisture vapor transmission plots-plastic closures.

plastic closures studied melamine and mineral filled plastic closures are more effective barriers to moisture vapor than phenolic resin closures. Removal torque values indicate that the difference in barrier efficiency of the three types of plastic closures is probably due to the "backoff," or slow unscrewing of phenolic closures. This is caused by the expansion of phenolic resin closures in a humid atmosphere. The use of melamine or mineral filled plastic closures decreases this "back off" tendency. One type of phenolic resin closure studied the Poly-Seal closure, did not backoff to any appreciable extent. This closure consists of a plastic cap with a polyethylene cone liner which molds itself around the sealing areas of the container. As the closure is applied the polyethylene liner takes the shape of the container finish. Removal torque values indicate

that metal closures show no appreciable tendency to "backoff" in a humid atmosphere.

Of the 16 liners investigated, polyethylene, aluminum foil plastic-wax saturated pulpboard, and red rubber liners were found to be the most effective moisture vapor barriers particularly when used in metal closures. A 0.040 in. solid polyethylene liner was considerably more effective than a 0.003 in. polyethylene extruded on paper liner, Fig. 2.

It is felt that studies of this type are important when considering problems of product stability, particularly of formulations that are adversely affected by moisture.

REFERENCE

- (1) *Chem. Eng. News* 34, 1126 (1956)

The Effect of Orally Administered Sodium Iodide and Sodium Iodate on Blood Sugar Response to Thiourea in Rats*

By ARTHUR H. MCCRELSH and DAVID E. MANN, Jr.

Sodium iodide and sodium iodate, when administered in drinking water for a period of two days as a 0.2 per cent solution, greatly diminished the hyperglycemia which normally follows the intraperitoneal injection of thiourea. The mechanism of action is not definitely known, but the protection may result from the prevention of epinephrine release, or the inhibition of hepatic glycogenolysis.

FOLLOWING THE OBSERVATIONS of DuBois, Holm, and Doyle (1), and, later, of Dubois, Hermann, and Erway (2) that, following the intraperitoneal injection of thiourea and its derivatives there occurs in adult rats a marked rise in blood sugar associated with increased hepatic glycogenolysis, several researchers have endeavored to prevent the hyperglycemia induced by these agents and thus, possibly, to uncover their mode of action and develop an antidote for the

toxic effects of pulmonary edema and pleural effusion, as seen in adult rats. The injection of iodide every four days has protected rats (3). Byerrum (4) and Mann (5) have shown that the oral administration of iodine and iodides, either as Lugol's solution, potassium iodide or potassium iodate, will similarly afford protection against the hyperglycemic response, and will enable the animal to withstand normally lethal doses.

This experiment is intended to determine the relative protective activity of sodium iodide and sodium iodate against the hyperglycemia following the intraperitoneal administration of ten milligrams per kilogram of body weight of thiourea to nonfasted, male, albino rats.

EXPERIMENTAL

Male rats of the Wistar strain were used exclusively in this experiment. The animals were placed in individual cages and were permitted to remain in these cages for periods of five to eight days before being used. All were fed Purina Lab Chow, and were given tap water *ad libitum* through glass tubes attached to drinking bottles until placed on a particular liquid diet. The same drinking bottles

* Received May 7, 1957 from Temple University School of Pharmacy, Philadelphia, Pa.
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Presented to the Society of Experimental Biology and Medicine, New York City meeting, May 1, 1957.

TABLE I.—EFFECT OF SODIUM IODIDE AND SODIUM IODATE ON BLOOD SUGAR RESPONSE TO THIOUREA IN NONFASTED, MALE, ALBINO RATS

Group	No of Animals	Treatment	Av Blood Sugar before Thiourea, mg/100 ml	Av Blood Sugar after Thiourea, mg/100 ml	Av Diff.	Av Change, %
1	27	Thiourea 10 mg/Kg. (controls)	113.63	183.48	69.85 (± 33.39)	63.67
2	12	Sodium Iodide <i>ad libitum</i> for two days, then thiourea	101.72	117.93	16.21 (± 13.62)	19.70
3	12	Sodium Iodate <i>ad libitum</i> for two days, then thiourea	112.20	132.63	20.53 (± 12.54)	18.90

were employed to deliver sodium iodide and sodium iodate solutions throughout the experiment. At the start of the experiment the animals were divided into three groups.

The rats in Group One, all nonfasting, male, albino rats, ranging in weight between 182 and 270 grams, were given tap water *ad libitum* as drinking water for two days, and served as controls in the experiment. After two days, the animals were bled from the tail, and blood glucose determined by the Folin-Malmros method (6). Immediately after withdrawing blood, a thiourea solution (10 mg./Kg. of body weight of a 0.5% aqueous solution) was injected intraperitoneally, and a second blood sample was taken after two and one-half hours. The blood glucose concentration was again determined by the Folin-Malmros method.

Group Two, consisting of nonfasted, male, albino rats, ranging in weight between 138 and 182 grams, was given a solution of sodium iodide (0.2%) *ad libitum* as drinking water for two days, whereupon blood was drawn from the tail, and the blood glucose level determined as above. Thiourea solution (10 mg./Kg. of body weight) was immediately injected intraperitoneally. After two and one-half hours a second blood sample was withdrawn and blood glucose determined.

Group Three consisted of nonfasted, male, albino rats, ranging in weight between 125 and 252 grams. These animals were treated identically as Groups One and Two, except that they received sodium iodate solution (0.2%) as drinking water *ad libitum* for two days. Blood samples were drawn from the tail both immediately before and two and one-half hours following the intraperitoneal injection of thiourea (10 mg./Kg. of body weight). Blood glucose was determined by the Folin-Malmros method as with all groups.

RESULTS

The rats in Group One, receiving no iodide or iodate therapy prior to the intraperitoneal injection of thiourea, showed an average blood sugar rise of 69.85 (± 33.39) milligrams per 100 milliliters of blood two and one-half hours following the injection.

The rats in Group Two, receiving a two-day, *ad libitum* administration of sodium iodide prior to an intraperitoneal injection of thiourea, showed an average blood sugar rise of 16.21 (± 13.62) milligrams per 100 milliliters of blood two and one-half hours after the injection.

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DISCUSSION

The rodenticidal property of thiourea and its derivatives, proposed by Richter (7), and tested on a controlled scale by MacKenzie and MacKenzie (8), and others in the early and middle forties, is a result of severe pulmonary edema and pleural effusion. Drinker (9) found an eighty-fold increase in lymph flow from the heart and lungs following an injection of alphanaphthylthiourea (ANTU) into rats. Although toxic doses vary greatly among strains of the same animal, and wild rats are much more resistant than laboratory animals, there can be no doubt of the toxicity of these agents.

It was DuBois, Holm, and Doyle (1) who first observed a rise in blood sugar following the administration of thiourea and its congeners, the hyperglycemia being associated with increased liver glycogenolysis; the onset, extent of activity, and duration being dependent upon the quantity given. DuBois, Hermann, and Erway (2) noted that the results were not altered by hypophysectomy, nor was there an inhibition of glycolysis or the oxidation of glucose.

Although the mechanism of action is uncertain and as yet no specific antidote has been found for thiourea, it has been observed that drugs which prevent or diminish the hyperglycemic response will also prevent or diminish the toxic effects. Griesbach, Kennedy, and Purves (3) have protected animals by the repeated injection of potassium iodide; and Byerrum (4) enabled rats to withstand many times the lethal dose by feeding the animals a diet rich in iodine, and by administering potassium iodide and iodine in drinking water. Mann (5) showed the protective action of potassium iodide and potassium iodate when administered *ad libitum* in drinking water. In this experiment the protective properties of sodium iodide and sodium iodate against the hyperglycemic response to thiourea have been demonstrated.

REFERENCES

- (1) DuBois, K. B., Holm, L. W., and Doyle, W. L., *Proc Soc. Exptl Biol Med*, 61, 102 (1946).
- (2) DuBois, K. B., Hermann, R. G., and Erway, W. G., *J Pharmacol Exptl Therap*, 89, 186 (1947).
- (3) Griesbach, W. E., Kennedy, T. H., and Purves, H. D., *Nature*, 154, 610 (1944).
- (4) Byerrum, R. U., *Proc Soc. Exptl Biol Med*, 62, 328 (1946).
- (5) Mann, D. E., *ibid*, 73, 657 (1950).
- (6) Folin, O., and Malmros, H., *J Biol Chem*, 83, 115 (1929).
- (7) Richter, C. P., *J Am Med Assoc*, 129, 927 (1945).
- (8) MacKenzie, J. B., and MacKenzie, C. G., *Proc Soc. Exptl Biol Med*, 54, 34 (1943).
- (9) Drinker, C. K., and Hardenbergh, E., *Am J. Physiol*, 156, 35 (1949).

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The rodenticidal property of thiourea and its derivatives, proposed by Richter (7), and tested on a controlled scale by MacKenzie and MacKenzie (8), and others in the early and middle forties, is a result of severe pulmonary edema and pleural effusion. Drinker (9) found an eighty-fold increase in lymph flow from the heart and lungs following an injection of alphanaphthylthiourea (ANTU) into rats. Although toxic doses vary greatly among strains of the same animal, and wild rats are much more resistant than laboratory animals, there can be no doubt of the toxicity of these agents.

It was DuBois, Holm, and Doyle (1) who first observed a rise in blood sugar following the administration of thiourea and its congeners, the hyperglycemia being associated with increased liver glycogenolysis; the onset, extent of activity, and duration being dependent upon the quantity given. DuBois, Hermann, and Erway (2) noted that the results were not altered by hypophysectomy, nor was there an inhibition of glycogenesis or the oxidation of glucose.

Although the mechanism of action is uncertain and as yet no specific antidote has been found for thiourea, it has been observed that drugs which prevent or diminish the hyperglycemic response will also prevent or diminish the toxic effects. Griesbach, Kennedy, and Purves (3) have protected animals by the repeated injection of potassium iodide; and Byerrum (4) enabled rats to withstand many times the lethal dose by feeding the animals a diet rich in iodine, and by administering potassium iodide and iodine in drinking water. Mann (5) showed the protective action of potassium iodide and potassium iodate when administered *ad libitum* in drinking water. In this experiment the protective properties of sodium iodide and sodium iodate against the hyperglycemic response to thiourea have been demonstrated.

REFERENCES

- (1) DuBois, K. B., Holm, L. W., and Doyle, W. L., *Proc. Soc. Exptl. Biol. Med.*, 61, 102(1946).
- (2) DuBois, K. B., Hermann, R. G., and Erway, W. F., *J. Pharmacol. Exptl. Therap.*, 89, 186(1947).
- (3) Griesbach, W. E., Kennedy, T. H., and Purves, H. D., *Nature*, 154, 610(1944).
- (4) Byerrum, R. U., *Proc. Soc. Exptl. Biol. Med.*, 62, 328 (1946).
- (5) Mann, D. E., *ibid.*, 73, 657(1950).
- (6) Folin, O., and Malmros, H., *J. Biol. Chem.*, 83, 115 (1929).
- (7) Richter, C. P., *J. Am. Med. Assoc.*, 129, 927(1945).
- (8) MacKenzie, J. B., and MacKenzie, C. G., *Proc. Soc. Exptl. Biol. Med.*, 54, 34(1943).
- (9) Drinker, C. K., and Hardenbergh, E., *Am. J. Physiol.*, 156, 35(1949).

A Study of the Effect of Lubricant and Fines on a Tablet Granulation*

By F. C. HAMMERNESS† and HERMAN O. THOMPSON‡

A series of six formulations was made in which the amount of lubricant added was a variable ranging from 0 to 5 per cent, and in which the amount of fines added was a variable ranging from 0 to 70 per cent. The rate of flow was measured, using a standard procedure which was modified for this experiment. A measurable effect was induced by the lubricant and the fines. The study was extended to determine whether the effects of lubricant and of fines could be demonstrated in the actual compression of the tablet. The results obtained indicate that some measurable effects can be noted. The physical properties evaluated were: weight variation, hardness, thickness, disintegration, and durability. The method of determining durability of tablets was devised for this study, while the other methods used were standard procedures.

A REVIEW OF THE LITERATURE (1-59) reveals that most of the investigation and research on tablet manufacture, with the exception of studies on tablet disintegration and tablet coating, have been conducted since 1945. This publication does not attempt to review the literature beyond providing a selected bibliography on tablet manufacture, inasmuch as references on tablet coating and disintegration are excluded beyond the citation of the two references (15, 16) which adequately cover these phases of studies.

The effects that fines and lubricant have upon a granulation are known empirically by many workers who have had experience in tablet manufacture, but there is no significant quantitative data to substantiate many of the statements made about them. Textbooks and bulletins are similarly deficient as to specific information which could be greatly beneficial to the beginner. This work was conducted to contribute quantitative findings as to these effects.

PRELIMINARY INVESTIGATION

The granulation used throughout this study consisted of lactose employing a 2% solution of liquid glucose U. S. P. as the binding and wetting agent. A 16-mesh sieve was used for screening, so that all granules that passed through a 16-sieve but were retained by a 40-sieve were considered a #16 granulation. The portion which passed through the 40-sieve was considered fines. These fines were analyzed with the following results:

40 to 60 mesh	28%
60 to 80 mesh	22%
80 to 100 mesh	15%
Less than 100	35%

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The above percentages were used in making "control fines" for the study. The #16 granulation contained no fines according to the above definition. Calculation of per cent fines added in each formulation was based upon the final total weight; that is, 10% fines was interpreted as 10 grams of fines and 90 grams of granulation.

Talc was used as a lubricant because of its general acceptance over the years and because of its economy and relative freedom from incompatibilities and adverse reactions. One gram of talc was used for each 100 grams of granulation, or 1% in this instance.

The density of the granulation determined in the conventional manner was found to be 1.505 as compared to 1.418 for lactose alone.

EXPERIMENTAL

To measure the effect of fines and lubricant upon tablet granulations, determinations were made both as to the rate of flow of the granulations and physical characteristics of the tablets compressed; furthermore a correlation was sought between the rate of flow and characteristics of the compressed tablets. A statistical analysis was performed on the data in an attempt to determine whether the results obtained could be expressed more quantitatively.

Flow Rate.—The rate of flow was measured by using a standard funnel and a measuring eup as described in A. S. T. M. Standard D-392-38(60). The stainless steel funnel measured $3\frac{21}{32}$ inches in diameter, $4\frac{1}{2}$ inches vertically from top to bottom, a $\frac{3}{8}$ inch inside diameter at the bottom and the side had a slope of 20 degrees from the vertical. The measuring cup was a glass cylinder with a capacity of 100 ml., calibrated to an accuracy of 0.5 ml. It measured 1.572 inches for inside diameter, and 3.144 inches for inside height.

The small end of the funnel was closed with the finger and a sample was poured into the funnel. The bottom of the funnel was quickly opened and the material was allowed to flow freely into the eup. After all of the material had passed through, the excess amount was leveled off with a spatula. The material in the eup was weighed to the nearest 0.1 gram. This amount of the granulation was placed in the funnel, and at the same instant that the bottom was opened a stop watch was started. The

watch was stopped as the last particle left the funnel. The time was recorded to the nearest 0.1 second. This same procedure was repeated five times for each sample and the average time in seconds was calculated.

The material was allowed to flow freely into the measuring cup without packing or tapping. Had the cup been tapped so as to increase the volume of granulation, theoretical values would have been approached more nearly, but in all probability the values would not have been more practical.

Test Granulations.—The samples consisted of the lactose granulation, "control fines," and lubricant. Granulations with 0, 10, 20, 30, 40, 45, 50, 55, 60, and 70% fines and no lubricant constituted one series of samples. The other series contained the same percentages of fines but lubricant was added in amounts of 1, 2, 3, 4 and 5 per cent. Based upon the theoretical investigation by Hoy (61), the fill ratio (Fr) and actual flow rate were determined. The fill ratio was the per cent of space occupied by the granules and was calculated as follows:

$$Fr = \frac{\text{Wt. of 100 ml. of granulation}}{\text{density} \times 100 \text{ ml.}}$$

The apparent volume was the space occupied by the granules and the void space between granules. The actual volume was the space occupied by the granules only. The apparent volume rate of flow was the volume of the cup (100 ml.) divided by the time in seconds. To obtain the actual number of milliliters that flowed per second, the apparent volume of flow was multiplied by the fill ratio. This actual flow rate was designated by the symbol Fa.

Results.—The data obtained as shown in Table I demonstrated that fines and lubricant do have a measurable effect upon the rate of flow with a general increase to a maximum followed by a decrease.

TABLE I.—ACTUAL VOLUME OF FLOW IN MILLILITERS PER SECOND (FA)

Fines, %	Lubricant, %					
	0	1	2	3	4	5
0	2.32	2.58	2.85	2.74	2.80	2.81
10	2.64	2.83	2.88	3.01	3.09	3.08
20	2.71	2.97	3.05	3.15	3.22	3.19
30	3.03	3.23	3.34	3.50	3.47	3.49
40	3.69	3.38	3.49	3.59	3.62	3.68
45	3.92	3.57	3.65	3.73	3.73	3.83
50	3.41	3.55	3.72	3.80	3.81	3.89
55	3.42	3.76	3.78	3.72	3.80	3.95
60	3.32	3.67	3.73	3.69	3.85	3.86
70	3.35	3.58	3.43	3.49	3.61	3.56

Tablet Compression.—Samples from the same formulations as those used in flow rate determinations were used for compression in an attempt to correlate the former to the actual compression of tablets. The tablet machine was kept in adjustment to constantly deliver a tablet of 300 mg. in weight and a hardness of 8 as measured with a Strong, Cobb hardness tester. With this particular formulation tablets containing 0, 1, and 2% lubricant could not be compressed satisfactorily to render an acceptable product.

Evaluation of the Compressed Tablets.—The criteria of evaluation were hardness, weight varia-

tion, thickness, disintegration, and durability. These are usual standards with the exception of durability. The criteria in the case of durability was a measurement of the loss in weight of 20 tablets due to frictional erosion. The tablets were placed in glass vials and allowed to shake for ten minutes in an Arthur shaker. The weight of the 20 tablets was recorded before and after shaking and the loss in weight recorded in mg. (11, 54-56).

TABLE II.—FORMULA 16 WITH 3, 4, AND 5% TALC

Fines, %	Av. Wt., mg.	Av. Thick- ness, mm.	Hard- ness, Strong Cobb Units	Av. Disinte- gration, sec.	Dura- bility Wt. Loss, mg.
3%					
0	317	4.28	6.9	24.2	15
10	333	4.44	7.1	22.3	25
20	311	4.18	5.85	27.1	20
30	319	4.31	7.95	25.8	25
40	344	4.49	8.5	31.8	15
45	349	4.64	7.35	25.2	40
50	a	a	a	a	a
55	a	a	a	a	a
60	a	a	a	a	a
70	a	a	a	a	a
4%					
0	321	4.17	10.2	46.3	15
10	314	4.25	5.9	26.7	25
20	504	4.21	5.0	22.7	35
30	314	4.29	5.7	24.5	40
40	332	4.43	6.35	26.9	40
45	344	4.50	6.65	28.2	20
50	338	4.53	5.25	23.6	45
55	322	4.39	5.15	22.4	20
60	332	4.45	6.2	25.8	25
70	331	4.44	5.7	24.2	30
5%					
0	320	4.37	4.3	21.4	25
10	329	4.40	4.75	28.8	30
20	327	4.40	5.05	29.6	35
30	331	4.36	5.6	29.6	25
40	329	4.37	5.2	26.9	25
45	320	4.34	5.2	26.5	25
50	326	4.37	5.95	26.8	20
55	320	4.29	6.6	29.9	15
60	330	4.45	5.05	23.7	35
70	322	4.35	5.05	24.1	40

a Satisfactory tablets could not be compressed in this range using this formulation.

Statistical Analysis.—The data collected in this study lends itself to statistical analysis, and the experiments were designed to facilitate such an analysis.

To measure the uniformity of each sample the reciprocal of the coefficient of variation was used; this is termed the coefficient of uniformity (C. U.).

On these C. U.'s the following analysis was performed:

(a) For each per cent lubricant group an equation was fitted relating the per cent fines to uniformity.

$$(\bar{y}/s) = C. U. = b_0 + b_1x_1 + b_2x_1^2$$

b_0 , b_1 , and b_2 are constants determined from the data.

x_1 = per cent fines x_1^2 = (per cent fines)²

In actual calculations it was easier to code first the

per cent fines to reduce the size of the numbers
This coding was done by the formulas

$$z_1 = (x_1 - \bar{x}_1), \bar{x}_1 = \text{mean per cent fines}$$
$$z_2 = (x_1 - \bar{x}_1)^2$$

The final form of the equation was then

$$C\ U = b_0 + b_1z_1 + b_2z_2$$

(b) For each fit made, a test of goodness of fit was made by the use of the statistic R^2 . R^2 is a measure of the strength of the relationship between per cent fines and uniformity. It may be thought of as varying from 0 to 1 where 0 is no relation and 1 is a perfect relation. It may also be thought of as $R^2 \times 100$ measuring the percentage of the variability in C. U.'s explainable by the different amounts of per cent fines, i.e., the "predictability" of uniformity knowing the per cent fines.

(c) Where R^2 was not larger than a value which could have occurred by chance (i.e., "nonsignificant") the conclusion is that there has not been a real relationship demonstrated.

(d) Three levels of "significance" were recognized: borderline, just short of the 5% level, significant, above the 5% value but short of the 1% value, and highly significant, exceeding the 1% value.

(e) Results were plotted for those measurements where R^2 was borderline or higher. For those results in which R^2 was less than borderline, the best estimate of the quantity is the mean value averaged over all per cent fines.

In the case of the durability and flow rate, uniformity was not the criterion, but the actual measurements themselves were related to the per cent fines in the same procedure as with the C. U.'s. Durability was measured as per cent weight loss, and flow rate was measured as ml. per second. See Figs. 1 and 2, and Table III for a summary of results.

DISCUSSION

As shown by the results in Table I the fines had a measurable and uniform effect upon the flow of a tablet granulation. The addition of fines increased the rate of flow to an optimum level, after which the rate began to decrease. A granulation of 100%

fines will not flow freely. With addition of lubricant to a granulation an appreciable improvement in the rate of flow was noted. The addition of lubricant beyond 2% did not substantially improve the rate of flow. In this case an optimum amount was not the criterion sought, but rather the minimum amount that could be used with practical efficiency. The addition of lubricant increased the rate of flow

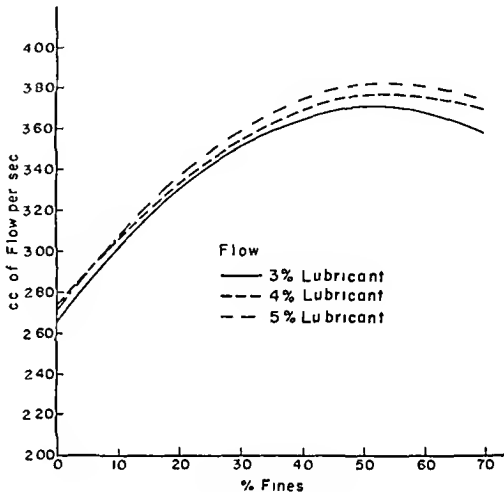


Fig. 1—The predicted rate of flow.

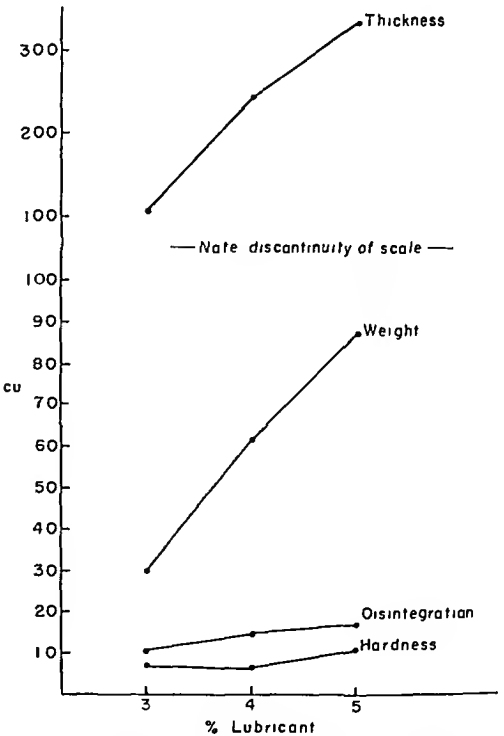


Fig. 2—Total mean coefficient of uniformity.

TABLE III— R^2 (R = MULTIPLE CORRELATION COEFFICIENT) SHOWING RELATIONSHIP BETWEEN PER CENT FINES EXPRESSED AS THE C. U. AND PHYSICAL PROPERTIES OF TABLETS FOR VARIOUS PERCENTAGES OF LUBRICANT

C. U. = (s/s)	Lubricant, %		
	3	4	5
Weight	0.40	0.21	0.32
Thickness	0.86 ^a	0.60 ^b	0.78 ^a
Hardness	0.15	0.67 ^c	0.14
Disintegration	0.13	0.64 ^c	0.35
Durability, wt loss, %	0.24	0.02	0.01
Flow Rate, ml./sec	0.96 ^a	0.99 ^a	0.92 ^a

^a Significance at the 1% level of probability.
^b Borderline significance.
^c Significance at the 5% level of probability.
Significance levels taken from Statistical Tables for Biological, Agricultural, and Medical Research by R. A. Fisher and G. Yates.

to a maximum, and then it began to decrease. It seems logical to assume that beyond the maximum point the lubricant gives the effect of too many fines.

A combination of lubricant and fines appeared to have a synergistic action upon the rate of flow, as the rate increased when both fines and lubricant were added. Again it could be noted that a maximum point was reached, which was the optimum ratio of fines and lubricant. The maximum lay between 50 and 60% fines regardless of the concentration of the lubricant.

When fines and lubricant have such uniform effects upon the flow of the granulation, the rate of flow for a given formula is indicative as to its behavior.

In general the results as noted in Table II seem to follow the same pattern as the flow rate. The degree of consistency was not as great as in the case of the flow rate determinations. The lubricant was shown to be increasingly effective in those cases where tablets could not be compressed satisfactorily, but did not apply to preventing binding and sticking in the die. The measurement of lubricant by the ease of ejection alone is not completely satisfactory nor does its measurement by rate of flow suffice.

Table III shows the relationship between per cent fines and the physical properties of the tablets that were measured for given percentages of lubricant. Thickness and flow rate were the only factors that appeared to have a real relationship throughout. This does not mean that the data for which no real relationship was demonstrated are valueless, but it does indicate that the extent of the effects if present were of the same order of magnitude as the chance factors.

The tests that were conducted on durability were not considered in the coefficient of uniformity or as predicted values of the same, as its R^2 value was so low as to be considered chance effect.

The most striking results are the measurements based upon the rate of flow (Fig. 1). The correlation as shown by these results approach near perfection, 1. In this case the R value is greater than 0.9 in all instances.

It has been shown that a lubricant and fines will have a direct effect upon the physical characteristics of tablets in that the variation of these two factors will have a direct effect on the rate of flow. It can be said that of the contributing factors, the lubricant and fines will be responsible for more than 90% of the variation in flow rate.

REFERENCES

- (1) Kehler, L. F., *THIS JOURNAL*, 3, 820, 937, 1062 (1954)
- (2) Higuchi, T., Arnold, R. D., Tucker, S. J., and Busse, L. W., *ibid.*, 41, 93 (1952)
- (3) Higuchi, T., Rao, N. A., Busse, L. W., and Swintosky, J. V., *ibid.*, 42, 194 (1953)
- (4) Higuchi, T., Nelson, E., and Busse, L. W., *ibid.*, 43, 344 (1954)
- (5) Nelson, E., Naqvi, S. M., Busse, L. W., and Higuchi, T., *ibid.*, 43, 506 (1954)
- (6) Higuchi, T., Clowe, L. N., and Busse, L. W., *ibid.*, 43, 685 (1954)
- (7) Clowe, L. N., Higuchi, T., and Busse, L. W., *ibid.*, 43, 718 (1951)
- (8) Nelson, E., Busse, L. W., and Higuchi, T., *ibid.*, 44, 223 (1955)
- (9) Münzel, K., and Kagi, W., *Pharm. Acta Helv.*, 29, 53 (1950)
- (10) Patel, B. C., and Guth, E. P., *Drug Standards*, 23, 37 (1955)
- (11) Spengler, O. H., and Kaelin, H. A., *Pharm. Acta Helv.*, 20, 219 and 239 (1945)
- (12) Arambulo, A. S., and Deardorff, D. L., *THIS JOURNAL*, 42, 690 (1953)
- (13) Arambulo, A. S., Fu, S. H., and Deardorff, D. L., *ibid.*, 42, 692 (1953)
- (14) Raff, A. M., Arambulo, A. S., Perkins, A. J., and Deardorff, D. L., *ibid.*, 44, 290 (1955)
- (15) Thompson, H. O., and Lee, C. O., *ibid.*, 34, 135 (1945)
- (16) Cooper, B. F., and Brecht, E. A., *ibid.*, 46, 520 (1957)
- (17) Newton, Philip, *Mfg. Chemist*, 23, 107 (1952)
- (18) Wolff, E. J., DeKay, G. H., and Jenkins, G. L., *THIS JOURNAL*, 36, 407 (1947)
- (19) Burlinson, H., *Pharm. J.*, 164, 437 (1950)
- (20) Burlinson, H., *J. Pharm. and Pharmacol.*, 6, 1035 (1954)
- (21) Nelson, Eino, *THIS JOURNAL*, 44, 494 (1955)
- (22) Smith, A. N., *Pharm. J.*, 167, 270 and 323 (1951)
- (23) Clarkson, R., *Drug & Cosmetic Ind.*, 66, 270, 408 and 524 (1950)
- (24) Chavkin, L., *ibid.*, 75, 466 and 559 (1954)
- (25) Weidemann, G. B., *Am. J. Pharm.*, 76, 30 (1904)
- (26) Silvers, J. A., *Am. Profess. Pharmacists*, 12, 444 (1946)
- (27) Denston, T. C., *J. Pharm. and Pharmacol.*, 6, 1067 (1954)
- (28) Smith, A. N., *Pharm. J.*, 156, 317 and 335 (1946)
- (29) Evers, N., *Quart. J. Pharm. and Pharmacol.*, 15, 6 (1942)
- (30) Dunnett, C. W., and Crisafio, R., *J. Pharm. and Pharmacol.*, 7, 314 (1955)
- (31) Little, A., and Mitchell, K. A., "Tablet Making," Northern Publishing Co., Liverpool, England, 1949
- (32) Arends, J., "Die Tablettenfabrikation und ihre maschinellen Hilfsmittel," Springer-Verlag, Berlin/Göttingen/Heidelberg, 1950
- (33) Silver, J. A., and Clarkson, R., "Manufacture of Compressed Tablets," F. J. Stokes Machine Co., Philadelphia, 1947
- (34) Woods, J. R., "Tablet Manufacture," J. B. Lippincott Co., Philadelphia, 1904
- (35) Foote, P. A., *Bull. University of Wisconsin*, 1, 163 (1928)
- (36) Beeler, E. C., and Gathercoal, E. N., *THIS JOURNAL*, 30, 56 (1941)
- (37) Swintosky, J. V., Riegelman, S., Higuchi, T., and Busse, L. W., *ibid.*, 38, 210, 308, and 378 (1949)
- (38) Brunauer, S., Emmett, P. H., and Teller, E., *J. Am. Chem. Soc.*, 60, 309 (1938)
- (39) Kimsey, C. E., and Thompson, H. O., to be published
- (40) Bikin, H., Jenkins, G. L., and DeKay, G. H., *THIS JOURNAL*, 38, 245 (1949)
- (41) Bikin, H., Jenkins, G. L., and DeKay, G. H., *ibid.*, 39, 441 (1950)
- (42) Patel, B. N., Jenkins, G. L., and DeKay, G. H., *ibid.*, 38, 247, and 250 (1949)
- (43) Sperandio, G. J., and DeKay, G. H., *ibid.*, 41, 245 (1952)
- (44) *Cosmetic and Drug Manual*, Glyco Products Co., Brooklyn, 1953
- (45) *Technical Information Bulletin*, #2, F. J. Stokes Machine Co., Philadelphia, 1955
- (46) Platt, R., and Silver, J. A., *Drug & Cosmetic Ind.*, 59, 39 (1946)
- (47) Dunnett, D., *Chemist and Druggist*, 78, 206 (1911)
- (48) Utz, F., *Pharm. J.*, 71, 42 (1903)
- (49) Gross, H. M., and Becker, C. H., *THIS JOURNAL*, 41, 158 (1952)
- (50) Batuyos, N. H., and Brecht, E. A., *ibid.*, 46, 524 (1957)
- (51) Smith, A. N., Private communication
- (52) McCallum, A., Buchter, J., and Albrecht, R., *THIS JOURNAL*, 44, 83 (1955)
- (53) Fillehorn, V. M., *Am. J. Pharm.*, 120, 239 (1948)
- (54) Smith, A. N., *Pharm. J.*, 163, 194, 227, 477 (1949)
- (55) Smith, A. N., *ibid.*, 164, 74 and 132 (1950)
- (56) Hasegawa, J., *J. Pharm. Soc. Japan*, 75, 480 (1955)
- (57) Bandelin, F. J., *Am. J. Pharm.*, 117, 124 (1945)
- (58) Aohi, M., and Fukuda, T., *J. Pharm. Soc. Japan*, 75, 878 (1955)
- (59) Nelson, E., *THIS JOURNAL*, 44, 435 (1955)
- (60) A. S. T. M. Standard D 392.38 American Society for Testing Materials, Philadelphia (1950)
- (61) Hoy, K. L., to be published

Correlation of Molecular Refraction with Structure in the Terpene Series*

By LUZ OLIVEROS-BELARDO† and PAUL J. JANNKE

Molecular refractions were determined for a number of terpenes and related compounds. Wherever optical exaltation occurred it was correlated with the structural configurations of the respective compounds. Optical isomers gave identical values while geometric isomers differed, with the *trans*-form giving the higher value. Isolated double bonds did not cause exaltation, but conjugated double bonds definitely increased the molecular refraction. This was particularly marked when an exocyclic bond was conjugated with one within the cycle. A double bond in conjugation with a three membered cycle gave higher exaltation than one in conjugation with a four membered cycle. The unique influence of the isopropyl group on the cyclic double bond nearest to it results in a marked change in molecular refraction.

TEXTBOOKS ON qualitative organic analysis mention molecular refraction as a valuable aid in the identification of organic compounds, yet this physical constant is seldom reported in the literature. Guenther (1) states that molecular refraction is a valuable constant frequently employed in determining the identity of isolates from the essential oils. However, in his own treatise, which is by far the most comprehensive work published on the volatile oils to date, molecular refraction is seldom listed among the physical constants of the terpenes and terpenoids.

Being replete with isomerism of all kinds, the terpenes present unusual problems in identification. This study was undertaken to disclose correlations which could be drawn between structural features of the isomers and variations in the basic molecular refraction.

DISCUSSION

Molecular refraction is fundamentally the sum of the individual atomic refractions. These values, however, show variation based on generic differences as is illustrated by oxygen in the hydroxyl, carbonyl, carboxyl, and ether types of linkages. The simple sum of the appropriate values may not agree with experimental results because of exaltation or depression resulting from inherent structural characteristics, such as a double bond conjugated with a cycle, a side chain or another double bond. Among isomers, subtle structural differences may account for wide variations in molecular refractions, and these variations when determined experimentally can be interpreted in terms of distinctive structural

habits. Correlation of such data should aid materially in the rapid identification of known terpenes and in projecting the structure of terpenes not yet known.

Experimental data compiled in this work was applied to the n^2 formula of Lorentz and Lorenz (2) which, it is claimed, is independent of temperature, pressure, and state of aggregation. In calculating the theoretical molecular refractions, the atomic refractions for the D-line determined by Brühl and Conrady (3) were found to give the most accurate results.

Understandably, the samples of terpenes and related compounds used in this study were necessarily of the highest purity attainable. Pure compounds of this type are not available commercially, therefore they were either isolated from the essential oils of high quality or were donated by laboratories of high repute.

Generally, samples were distilled at reduced pressure immediately prior to use. For the larger samples, a Todd precise fractionation assembly was used, at various reflux ratios. When the volumes of samples were of the order of 2.5 ml and less, small scale fractionating equipment¹ was employed. Isolates were held under nitrogen in tightly closed containers. They were maintained in a constant temperature bath for about twelve hours prior to being used in measurements. The prisms of the refractometer² were maintained at 25° by means of a circulating water-temperature regulator. Whenever possible, specific gravities were determined in a 10-ml. Geissler pycnometer, for greater accuracy. When only small volumes of samples were available, Sprengel-Ostwald type pycnometers with capacities ranging from 0.1 to 5.0 ml were employed. These, too, were maintained at a constant temperature by keeping them in a closed container which was immersed in the water bath.

The data which are recorded were checked repeatedly in order to be assured of their accuracy. The only substance which did not behave consistently was *d*-limonene. That which was produced by the fractionation of sweet orange oil (cold-pressed), and repeatedly distilled from metallic sodium, appeared to be identical with a sample furnished by the Naval

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² Bausch and Lomb Optical Co., Type 33-45-56

Stores Station. On the other hand, *d*-limonene isolated from lemon oil boiled at only 0.1° less than the *d*-limonene from orange oil, yet the differences between their specific gravities, refractive indices, and optical rotations were very great. Based on the low rotation of the *d*-limonene from lemon oil, it might be concluded that some *l*-limonene could be present, however *l*-limonene has never been reported to occur in citrus oils. The alternative explanation is that in addition to *d*-limonene of conventional configuration lemon oil may contain the *d*- and *l*-forms of the strainless ring isomers (the chair and the boat forms) whose angular rotation, depending upon their proportions, could depress that of *d*-limonene of planar configuration. It is of significance to note that the refractive index (1.4725) reported for *d*-limonene by some investigators (4) was attained only after the limonene from lemon oil stood for sixteen days under ordinary conditions of storage.

EXPERIMENTAL

Samples of hydrocarbons obtained from the various sources³ were carefully prepared for physical measurements (see Discussion) which were applied in the calculation of molecular refractions (Table I)

CONCLUSIONS

An analysis of the data given in Table I affords the following conclusions.

1. Based on the figures referred to by past investigators as to the range of normal and anomalous refractions,⁴ the results of this investigation show that normal molecular refraction was shown by *trans-p*-menthane, *cis-p*-menthane, 3-*p*-menthene, limonene, dipentene, terpinolene, *p*-cymene, and α -pinene. Those that gave exaltation were 3,8-*p*-menthadiene, α -terpinene, *d*- α -phellandrene, β -pinene, and sabinene.

2. The identical molecular refraction obtained for limonene from oil of orange (IV, Table I) and dipentene (V, Table I) shows that optical isomerism introduces no difference in molecular refraction.

3. It will be noticed that even within the group of isomeric compounds showing normal molecular refraction, the effect of structural variation is demonstrated, in that there exist in some of them a difference in degree of normal refraction. Thus, *trans-p*-menthane (I, Table I) gave a higher value than its isomer, *cis-p*-menthane (II, Table I). The foregoing results agree

with the findings of Bruhl who showed that optical isomers give the same molecular refraction while geometric isomers differ, the *trans*-form giving the higher value.

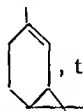
4. Since the molecular refraction of limonene, dipentene, and terpinolene (IV, V, and VIII, Table I) fall within the range of normal values, the isolated double bonds in each of these compounds did not cause optical anomaly.

5. Of the bicyclic terpenes used in this investigation, β -pinene and sabinene showed marked exaltations. α -Pinene gave the highest refraction among those that gave normal refractions.

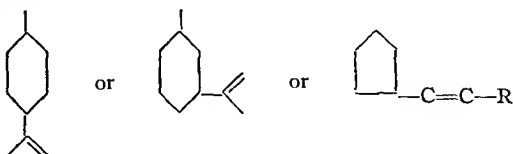
6. Conjugated double bonds definitely raised molecular refraction. 3,8-*p*-Menthadiene (VI, Table I), α -terpinene (VII, Table I), and *d*- α -phellandrene (IX, Table I) all showed optical exaltation. Of these hydrocarbons, 3,8-*p*-menthadiene gave the highest exaltation, indicating that an exocyclic double bond conjugation with a double bond inside the ring produces high exaltation.

p-Cymene (X, Table I), with its three double bonds in conjugation, became an exception. The sudden drop from the marked exaltation of α -terpinene to the normal refraction of *p*-cymene is consistent with reports that the benzene ring does not show optical exaltation.⁵

7. The exaltation of β -pinene and sabinene and the almost low exaltation of α -pinene show that a double bond conjugating with a cycle could cause exaltation. From the result obtained for sabinene, and from reports in litera-

ture (5) for Δ^4 carene, , the location of the

cycle that is in conjugation with the double bond is immaterial, i. e., it may be an inner or an outer cycle. As to whether or not this property holds true only for bicyclic compounds cannot be held conclusive until measurements are made for compounds having the following types of configuration



where a double bond lies in conjugation with a cycle in a monocyclic compound.

8. Comparing the exaltations obtained for

³ The authors gratefully acknowledge the receipt of samples of hydrocarbons for this study. The Hercules Powder Co., Wilmington, Del., kindly furnished *cis-p*-menthane, 3-*p*-menthene, 3,8-*p*-menthadiene, α -terpinene and terpinolene. The Naval Stores, U S D A, Olustee, Fla., kindly supplied *trans-p*-menthane, *d*-limonene, and α -pinene.

⁴ A difference of $+0.03$ to $+0.26$ was considered "normal" refraction, $+0.34$, "little exaltation" $+0.5$, "exaltation", and above $+0.5$, "marked exaltation".

⁵ Measurements for purified toluene and purified benzene were made as a side line to this work. It was observed that both of these hydrocarbons did not show exaltation.

TABLE I—STRUCTURAL CONFIGURATION AND MOLECULAR REFRACTION

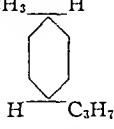
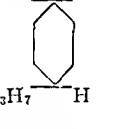
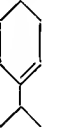
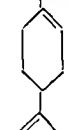
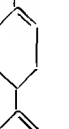
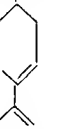
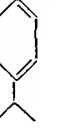
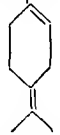

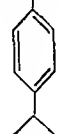



	Compounds	n_D^{25}	d_{25}^{25}	Molecular Refraction		
				Calcd	Found	Difference
I	Trans- <i>p</i> -menthane (C ₁₀ H ₂₀) 	1.4364	0.7962	46.03	46.08	+0.05
II	Cis- <i>p</i> -menthane (C ₁₀ H ₂₀) 	1.4412	0.8085	46.03	45.81	-0.22
III	3- <i>p</i> -Menthene (C ₁₀ H ₁₈) 	1.4501	0.8109	45.64	45.81	+0.17
IV	<i>d</i> -Limonene (C ₁₀ H ₁₆)					
	From { Lemon Oil ^a	1.4718	0.8492	45.24	44.90	-0.34
	Orange Oil ^b	1.4706	0.8414	45.24	45.20	-0.04
	Naval Stores ^c	1.4709	0.8413	45.24	45.24	-0.00
						
V	Dipentene (C ₁₀ H ₁₆) 	1.4708	0.8420	45.24	45.20	-0.04
VI	3,8- <i>p</i> -Menthadiene (C ₁₀ H ₁₆) 	1.4902	0.8550	45.24	46.08	+0.84
VII.	α-Terpinene (C ₁₀ H ₁₆) 	1.4759	0.8373	45.24	45.86	+0.62

TABLE I (Continued)

Compounds	n_D^{25}	d_{25}^{25}	Molecular Refraction		
			Calcd.	Found	Difference
VIII. Terpinolene (C ₁₀ H ₁₆) 	1.4877	0.8632	45.24	45.42	+0.18
IX. <i>d</i> - α -Phellandrene ^d (C ₁₀ H ₁₆) 	1.4691	0.8323	45.24	45.58	+0.34
X. <i>p</i> -Cymene ^e (C ₁₀ H ₁₄) 	1.4878	0.8584	44.84	45.02	+0.18
XI. α -Pinene (C ₁₀ H ₁₆) 	1.4636	0.8577	43.53	43.79	+0.26
XII. β -Pinene ^f (C ₁₀ H ₁₆) 	1.4733	0.8650	43.53	44.19	+0.66
XIII. Sabinene ^g (C ₁₀ H ₁₆) 	1.4641	0.8424	43.53	44.61	+1.08

^a Limonene fraction b p 66.3°, at 15 mm, $[\alpha]_D^{25} + 86^\circ 36'$ ^b Limonene fraction b p 66.4°, at 15 mm; $[\alpha]_D^{25} + 122^\circ 24'$ ^c B p 66.4°, at 15 mm; $[\alpha]_D^{25} + 122^\circ 24'$ ^d Distilled from Manila elemi oil, b p 61.0°, +117°2'^e Commercial terpene-free *p*-cymene distilled at 15 mm yielded fraction 53.5–54.0°, which was redistilled from metallic sodium at 760 mm, b p 174.0°^f From Canadian fir oil, b p 165–165.5°, at 760 mm^g From savin oil, b p 66.5°, at 30 mm


β -pinene (XII, Table I) and sabinene (XIII, Table I), and taking into consideration the reported exaltation for Δ^4 carene, it appears that an ethenoid linkage conjugating with a three-

membered ring produces exaltation higher than that produced by conjugation with a four-membered ring.

9. The exalting effect of an ethylenic bond

outside but attached to the ring was demonstrated in terpinolene (VIII, Table I), β -pinene (XII, Table I), and sabinene (XIII, Table I). The exaltation due to conjugation between an ethenoid linkage and a cycle was already explained in one of the preceding paragraphs. However, α -pinene, with its double bond conjugating with a cyclobutane ring does not show this effect because its difference of 0.256, although the highest obtained among the normal refractions in this work, does not come up within the range of what can be called true exaltation. But in shifting the double bond to the outer part of the ring, as in β -pinene, there is produced marked exaltation. Therefore, the position of the double bond, more than the conjugation between double bond and cycle, seems to be responsible for the marked exaltation. The observed molecular refraction for terpinolene supports this observation. While the molecular refraction for terpinolene lies within the normal values, it is far above those for dienes where an exocyclic ethenoid linkage is removed from the ring as in limonene and dipentene.

The high exaltation of sabinene could probably be interpreted as a function of the presence of two highly influential conditions for exaltation; namely, the ethenoid bond attached to the nucleus, and the same, conjugating with a three-membered ring. Further studies along this line would have been worthwhile had there

been some α -thujene, , available for comparison and verification.

10. The isopropyl group seems to exert an exalting influence on the cyclic double bond near-

est to it. Although the measurements for benzene and toluene were done primarily for verification of the nonexalting effect of the benzene ring, the results of the analysis tend to show the effect of the isopropyl group on molecular refraction in the *p*-menthene series. Benzene gave a difference of -0.248 , and toluene -0.1268 , or a difference of -0.1212 between them. But from toluene to *p*-cymene, a sudden rise of $+0.3077$ difference was noted. Since the essential structural difference between toluene and *p*-cymene lies in the isopropyl radical, it is evident that the sudden rise in difference must have been brought about either by the isopropyl group *per se*, or by a combined effect of the presence of the group and its influence on the neighboring double bond.

Results of measurements for α -phellandrene and α -terpinene lend a strong support to the combined effect, since both of them contain the isopropyl group. They also have a system of conjugated double bonds inside the cycle. The 1-2 position of one double bond is common to them. The only apparent difference that could create the wide gap between their molecular refractions is the position of their respective second double bond; that of α -terpinene being nearest to the isopropyl group. By the same token, the marked exaltation of 3,8-*p*-menthadiene may be due, not only to the system of conjugation occurring between an exocyclic and cyclic bonds, but also to what may be a unique influence of the isopropyl group on the double bond in position 3-4.

REFERENCES

- (1) Guenther, E., "The Essential Oils," Vol. I, D. Van Nostrand Co., New York, 1948, p. 247.
- (2) Lorentz, H., and Lorenz, L., *Wied. Ann.*, 9, 641(1880).
- (3) Guenther, E., "The Essential Oils," Vol. I, D. Van Nostrand Co., New York, 1948, p. 248.
- (4) Padmanabhan, R., and Jatkari, S., *J. Am. Chem. Soc.*, 57, 334(1935).
- (5) Guenther, E., "The Essential Oils," Vol. II., D. Van Nostrand Co., New York, 1949, p. 52.

Studies on the Effects of Ephedrine in the Presence of Cocaine*

By PHILLIP V. HAMMOND†

The pressure response of ephedrine was found to be reduced in anesthetized dogs by the previous administration of cocaine, as has been reported in earlier works. It was determined that this reduction was not due to tachyphylaxis. A definite relationship between dosage of cocaine, time allowed for the animal to become cocainized, and a lessening to the response of ephedrine were observed. Similar effects were also ascertained in studies on spleen volume. The action of the two drugs on cardiac output was determined by means of the radioactive isotope dilution method using I^{131} .

IT HAS BEEN OUR EXPERIENCE to note that ephedrine in the presence of cocaine does not produce a rise in the blood pressure of the anesthetized dog within ranges usually observed with a given dose of ephedrine. Indeed on one or two occasions the rise in blood pressure induced by ephedrine in the cocainized animal was so slight that it was insignificant.

At one time ephedrine was thought to have a mechanism of action similar to epinephrine (1), i. e., acting through the same receptor mechanism. However, this does not explain the many unrelated actions such as tachyphylaxis, central nervous system stimulation, prolonged action, and desensitization by cocaine.

In 1928 Chen (2), using a small dose of cocaine (10 mg.), reported the effects of cocaine on the pressor action of ephedrine. Chen reported that the rises of blood pressure produced were within normal ranges. However, Tainter (3) in 1929 reported that cocainization of cats and dogs prevented or greatly decreased the pressor, cardiac, and respiratory responses to ephedrine and simultaneously sensitized the pressor and cardiac responses to epinephrine. Tainter also reported that he obtained the desensitization to ephedrine and also the sensitization to epinephrine regardless of the type of anesthesia and also in the absence of anesthesia.

In 1933 Holck (4) explained a method for demonstrating the difference between ephedrine

and epinephrine blood pressure response after cocaine. In 1953 Shinyo (5) reported that cocaine was rather synergistic with epinephrine but to other sympathomimetic amines it was rather antagonistic. Despite the effects of many investigators who have contributed to the vast amount of literature on this drug, the exact mechanism of action has not been definitely established. This paper, based largely on pilot experiments, is concerned with further elucidation of the mechanism of action of ephedrine on the cardiovascular system in the presence of cocaine.

EXPERIMENTAL

Pressure Response.—Throughout the pressor experiments a total of twelve mongrel dogs of both sexes were used. The trachea and the carotid artery were isolated and cannulated. Blood pressure was recorded by means of a mercury manometer. Respiratory changes were recorded by means of a tambour. A 4% solution of cocaine hydrochloride in varying doses was injected subcutaneously and a 1% solution of ephedrine hydrochloride in doses of 0.3 mg./Kg. was injected into the femoral veins of dogs under sodium pentobarbital anesthesia (30 mg./Kg.). The procedure was to ascertain the normal blood pressure after the animal had become stabilized, inject ephedrine, and after a suitable interval of at least forty-five minutes and the blood pressure had returned to normal, cocaine was injected. Twelve minutes were allowed for the animal to become cocainized. At the termination of the cocainization period a second injection of ephedrine was administered. This procedure was repeated three or four times. Electrocardiographic changes were taken with a Sandborn Viso-Cardiette using standard limb leads.

Isolated Heart.—Cocaine and ephedrine are known to be antagonistic on the heart. Several experiments were carried out on the isolated rabbit heart using the Langendorff and Anderson pump (6, 7). The purpose was to observe whether or not this antagonism could be demonstrated in the isolated heart preparation. The animal was sacrificed by a sharp blow on the back of the neck. The heart was quickly removed and placed into the chamber. Here it was oxygenated and perfused with a modified Ringer's solution at a constant temperature of 37.5°. After the normal amplitude, the normal rate of contraction, and the perfusion rate had been determined, the two drugs were perfused through the preparation at definite intervals. Cocaine was introduced in doses of 4 mg. whereas ephedrine was employed in doses ranging from 1.5 mg. to 15 mg.

Spleen Volume Change.—Ephedrine in the presence of cocaine was tested on splenic volume changes by opening the abdomen of small anesthetized dogs,

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The author wishes to thank Dr. Linwood Rayford for his assistance in the cardiac output studies.

Anticonvulsant Activity of Pyrazole and of Pyrazole with Aliphatic Substitutions at the Three Position*

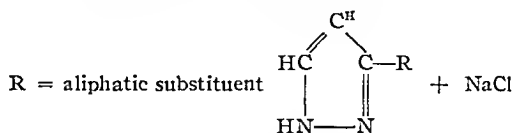
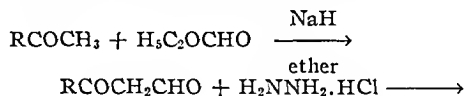
By JOHN E. OWEN, Jr.,† EDWARD E. SWANSON,‡ and DONALD B. MEYERS§

Pyrazole and a series of 3-*n*-aliphatic substituted pyrazoles have been shown to have anticonvulsant activity in rats and mice. In this study, extension of the length of the aliphatic chain up to four carbons increased the acute toxicity and anticonvulsant activity of these compounds. Further lengthening of the substituents decreased the toxicity and activity. The compounds, as anticonvulsants, were found to be more active in rats than in mice.

AMONG A SERIES of nitrogen-containing five-membered heterocycles investigated for central nervous system activity by Gibson, *et al.* (1), some substituted pyrazoles were found to protect rats from convulsions induced by electroshock and pentylenetetrazol (Metrazol). This finding has added another type of compound to the group of drugs classified as anticonvulsants. A survey of the literature has revealed relatively little information on the pharmacology of pyrazole compounds. Those on which data are available have been reported to exert a variety of actions, including central nervous system stimulation and depression, diuresis, and some muscletropic action on smooth muscle. The depression has been characterized by varying degrees of sedation, analgesia, and antipyresis. However, Gibson, *et al.* (1), appear to have been the first to report on the anticonvulsant activity of pyrazoles.

Because certain of these pyrazoles with substitutions at the 3-position were found to possess significant anticonvulsant activity, further study of a homologous series substituted at that position seemed indicated. The investigation reported here was limited to an examination of the influence of straight-chain aliphatic substitutions at the 3-position on the acute toxicity and anticonvulsant properties of pyrazole.

studied were: pyrazole, 3-methylpyrazole, 3-*n*-propylpyrazole, 3-*n*-butylpyrazole, 3-*n*-amylpyrazole, and 3-*n*-nonylpyrazole. The compounds were prepared by the following general reaction:¹



These compounds, with the exception of pyrazole, are clear, yellow oils. Pyrazole is a white crystalline substance which melts at 70°. All compounds were relatively insoluble in water.

Methods.—The compounds were administered orally as gum acacia emulsions. Rats of both sexes of a Wistar derived strain, weighing 90–140 Gm.; and mice of both sexes of the Bittner-A strain, weighing 15–30 Gm., were employed for the assays.

Acute Toxicity.—The median lethal dose (LD₅₀) with standard error (S. E.) was determined for each compound in both the rats and the mice. The animals were observed for lethal effects over a period of seventy-two hours.

Anticonvulsant Activity.—Anticonvulsant activity was evaluated by the maximal electroshock seizure test (M. E. S.) of Toman, *et al.* (2), and by the subcutaneous Metrazol seizure test described by Swinyard (3). A dose response curve of anticonvulsant activity at a predetermined time of peak activity was established for each compound on each of the procedures. The median lethal and median effective (ED₅₀) doses with standard errors were computed by the Bliss method (4).

RESULTS

The data obtained from the assays of these pyrazole compounds have shown that pyrazole itself has anticonvulsant activity in rats and mice and that normal aliphatic substitutions at the 3-position quantitatively altered this activity (Tables I, II, and III).

the 3-*n*-butylpyrazole to have the optimal activity and safety margin on all procedures except the subcutaneous Metrazol test in mice. The 3-*n*-butyl compound appeared to be the most active member of the series but the 3-*n*-propylpyrazole had a slightly better T. I.

At the doses used to determine acute toxicities, the pattern of depression produced in the rats and the mice was the same for all of the compounds. The animals became ataxic with general muscle weakness followed by loss of righting reflexes and slowed respirations. They became comatose with pronounced cyanosis and death resulted from respiratory failure.

The onset of peak activity (Table IV) was more rapid and the duration of peak activity shorter in

mice than in rats. However, in rats, it was observed that at the end of peak activity time, the activity fell off very rapidly with all of the compounds except the 3-*n*-nonylpyrazole. In mice, for the most part, there was a gradual decline in the activity after the end of peak action. In Table IV the duration of activity was estimated as that interval of time, including the peak time duration, at which no less than 25% of the animals were protected from convulsions.

All compounds proved more active as anticonvulsants in rats than in mice. The 3-*n*-propylpyrazole, and 3-*n*-amylpyrazole were more toxic in rats than in mice. The T. I.'s were shown to be more favorable in rats than in mice.

DISCUSSION

The compounds were tested in both rats and mice to determine whether a species difference existed. Swinyard, *et al.* (5), considered that either animal is suitable for this type of testing but that it is desirable for a more definitive comparison to use both species. Furthermore, unless the compounds being tested are chemically related to agents known to have antiepileptic effects, these assays may be seriously limited in predicting any clinical efficacy unless at least two species are used. In this evaluation of 3-*n*-aliphatic substituted pyrazoles as anticonvulsants, the qualitative results were similar for

TABLE I.— $LD_{50} \pm$ S. E. OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES IN RATS AND MICE

Substituent	$LD_{50} \pm$ S.E., mg./Kg.	
	Rats	Mice
Hydrogen	1734.0 \pm 109.2	1456.0 \pm 96.1
Methyl	1312.0 \pm 78.7	885.5 \pm 59.3
Propyl	615.5 \pm 52.3	778.0 \pm 38.1
Butyl	509.3 \pm 25.5	543.7 \pm 24.5
Amyl	522.9 \pm 41.3	637.7 \pm 27.4
Nonyl	2695.0 \pm 207.5	1078.0 \pm 87.3

TABLE II.— $ED_{50} \pm$ S. E. AND T. I. OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES AGAINST M.E.S.

Substituent	Rats		Mice	
	$ED_{50} \pm$ S.E.	T. I.	$ED_{50} \pm$ S.E.	T. I.
Hydrogen	89.32 \pm 8.22	19.48	284.10 \pm 11.60	5.13
Methyl	66.99 \pm 6.79	19.88	173.90 \pm 6.80	5.12
Propyl	32.86 \pm 3.00	19.22	101.50 \pm 4.50	7.70
Butyl	14.70 \pm 2.06	36.35	66.99 \pm 4.42	8.23
Amyl	37.33 \pm 3.88	14.11	79.49 \pm 5.17	8.06
Nonyl	48.09 \pm 4.81	56.15	86.24 \pm 8.50	12.53

TABLE III.— $ED_{50} \pm$ S. E. AND T. I. OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES AGAINST METRAZOL INDUCED SEIZURES

Substituent	Rats		Mice	
	$ED_{50} \pm$ S. E.	T. I.	$ED_{50} \pm$ S. E.	T. I.
Hydrogen	98.47 \pm 11.32	17.70	377.6 \pm 19.2	3.89
Methyl	131.80 \pm 9.70	10.84	236.2 \pm 27.6	3.75
Propyl	76.58 \pm 13.02	8.09	138.7 \pm 8.6	5.64
Butyl	38.10 \pm 6.48	13.40	109.5 \pm 7.7	4.98
Amyl	71.93 \pm 7.34	7.35	226.0 \pm 22.7	2.82
Nonyl	141.90 \pm 20.60	19.11	327.3 \pm 17.3	3.30

TABLE IV.—PEAK TIME AND DURATION OF ANTICONVULSANT ACTIVITY OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES

Substituent	Rats			Mice		
	Dose, mg./Kg.	Peak Time, min.	Duration, min.	Dose, mg./Kg.	Peak Time, min.	Duration, min.
Hydrogen	70	300-360	^a	160	240	^a
Methyl	80	300-360	^a	250	90	300
Propyl	40	60-120	^a	125	15-30	200
Butyl	25	30-120	^a	80	15-45	75
Amyl	40	30-120	^a	80	15-60	^a
Nonyl	50	60-120	180	100	15-45	90

^a The drug activity at onset of action rose very rapidly to the peak and fell off rapidly after peak time.

exposing the spleen and placing it in an oncometer. The oncometer was made airtight by covering the opening with gauze which had been impregnated with petrolatum. Volume changes were registered by a tambour and made to record on a kymograph. Cocaine in doses of 15 mg./Kg. was injected subcutaneously. Ephedrine in doses of 0.3 mg./Kg. was injected intravenously.

Cardiac Output.—Cardiac output studies were conducted by means of the radioactive isotope dilution method (8) using iodinated (I^{131}) human-serum albumin. Twenty cubic centimeters of Lugol's solution were administered orally to prevent the thyroid from picking up the radioactive iodine. Sodium pentobarbital 30 mg./Kg. was used as the anesthetic agent. The jugular vein and femoral artery were isolated and cannulae and Courmand needle were inserted. Iodinated (I^{131}) human-serum albumin was injected in doses of 30 microcuries into the jugular vein. The dilution technique employed was to dilute 1 cc. of radioactive iodinated human-serum albumin to 5 cc. with normal saline. Of this dilution, 0.1 cc. was diluted to 10 cc. One cubic centimeter of the aliquot was used as a control. The volume of the dose was 4.4 cc. The dilution curve was obtained by plotting the radioactivity of the blood flowing over the detector as a function of time

RESULTS

Pressor Response.—In all experiments, and with doses of cocaine as little as 4 mg./Kg., the pressor response to ephedrine was reduced. Represented values of this diminution are presented in Fig. 1. The reduced pressor response of ephedrine in the cocaineized animal was found to increase with increased doses of cocaine against a standard dose of ephedrine. A dose of 30 mg./Kg. of cocaine prevented 89.65% of the pressor response to cocaine. This was greater than that obtained by Hamet (9). Hamet reported that ephedrine alone increased 129%, and that ephedrine after cocaine only increased the blood pressure on the average of 71%, a reduction of 58% of the normal pressor response. In one experiment ephedrine did not increase the blood pressure in the cocaineized animal. However, these results were not reproducible. In one experiment cocaine was injected into the animal prior to the administration of ephedrine. This was followed

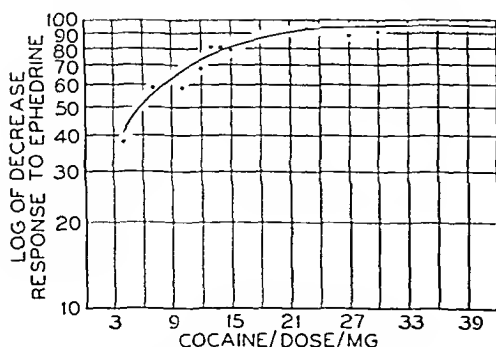


Fig. 1—Diminution of pressor response to ephedrine in the cocaineized animal.

by a dose of ephedrine at the end of twelve minutes. In this instance the inhibition of the pressor effect which occurred when a single dose of ephedrine was administered was less than in experiments when cocaine followed the first dose of ephedrine and subsequent doses of ephedrine were administered at definite intervals. For example at a dose of 15 mg./Kg. cocaine alone produced a 20% diminution in pressor response to ephedrine; whereas cocaine at a dose of 15 mg./Kg. produced a 78% diminution in pressor response to ephedrine when administered to an animal who had received one dose of ephedrine. An interval of one hour or more between doses of ephedrine (0.3 mg./Kg.) prevented tachyphylaxis.

Electrocardiographic changes revealed that immediately after the first dose of ephedrine there was apparently no change in heart rate. However, five minutes after the administration of ephedrine there was an average increase in heart rate of 50%. Because of the increase the *T* and *P* waves became closer. The change in voltage or amplitude was insignificant, and the *S* wave became depressed. Five minutes after the administration of cocaine, the heart rate remained the same, and the amplitude remained the same; however, the *S* wave became more depressed. Ten minutes after the administration of cocaine, the *S* wave appeared further depressed; the heart rate remained the same, and the amplitude remained the same; however, the *S* wave became more depressed. The maximum effect after the administration of cocaine revealed that the *S* wave was further depressed; the heart rate remained the same; and the *T* wave appeared more prominent.

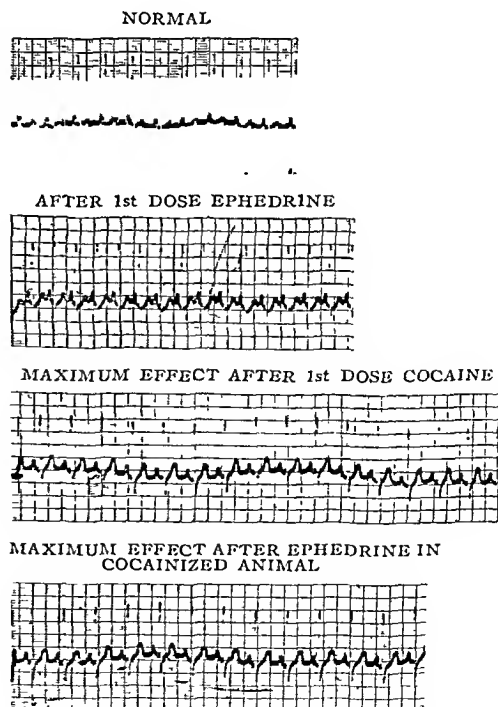


Fig. 2—Cardiographic changes of ephedrine in the presence of cocaine.

Immediately after the administration of ephedrine in the cocaineized animal, the heart rate remained the same; the *S* wave was still depressed; and the *T* wave became less prominent than when seen following cocaine. Five minutes after the second dose of ephedrine in the cocaineized animal, the amplitude and the rate remained the same; however, the *S* wave depression was somewhat less.

The maximum effect of ephedrine in the cocaineized animal revealed a decrease in heart rate, prominent depression of the *S* wave, a slight fall in voltage or amplitude, and no changes in rhythm.

Isolated Rabbit Heart.—Ephedrine did not afford protection against cocaine. However, with increased doses of ephedrine, the response to the depression of cocaine was somewhat lessened. The toxic levels of ephedrine, however, were soon reached, which levels resulted in a marked decrease in rate and response similar to that induced by cocaine.

Splenic Volume Changes.—As seen in Fig 3, ephedrine alone produced not only a marked increase in blood pressure, but also a similar increase in contraction of the spleen. Cocaine produced a slight degree of contraction; however, ephedrine in the presence of cocaine produced no significant change in splenic contraction.

Cardiac Output.—Cardiac output studies are still in progress. However, there appears to be a definite trend which may be typified by the following experiment (see Table I).

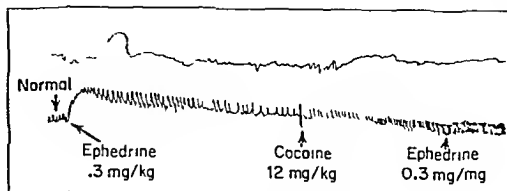


Fig 3—Splenic contractions

prior to the administration of cocaine, the *S* wave appeared depressed and the *T* wave appeared more prominent. The maximum effect of ephedrine in the cocaineized animal showed a substantial drop in heart rate from that seen after ephedrine alone, this was accompanied by a slight fall in amplitude. There was no change in rhythm; however, there was a marked depression of the *S* wave. This latter finding is in keeping with the increase in cardiac output.

3. Splenic volume changes (i.e., contraction) accompanied the increase in blood pressure induced by ephedrine. This splenic contraction was abolished by the prior administration of cocaine.

4. Ephedrine increases the cardiac output.

TABLE I

	Normal	Ephedrine, 0.3 mg./Kg.	Ephedrine 0.3 mg./Kg. in the Cocainized Animal
Cardiac Output	3.63 L./Min.	5.77 L./Min.	5.33 L./Min.
Cardiac Index	4.5 L./Min./M ²	7.2 L./Min./M ²	6.67 L./Min./M ²
Total Peripheral Resistance, Gm./dynes/ cm. ²	2,564	1,605	1,738
Heart Rate	170	180	175

From Table I, it can readily be seen that ephedrine substantially produces an increase in cardiac output. However, the reduction in cardiac output induced by ephedrine in the presence of cocaine was not so great as expected. Further, it was somewhat surprising that the total peripheral resistance was lessened. Also the increase in heart rate was not so great as that revealed by the electrocardiographic method. More cardiac output studies are planned.

SUMMARY AND CONCLUSIONS

1. Experiments have been described demonstrating reduced response of cocaineized animals to standard doses of ephedrine. Under proper conditions this reduction amounted to as much as 90 per cent. It was determined that tachyphylaxis was not responsible for the diminution in response to ephedrine in the cocaineized animal after an interval of one hour or longer.

2. Electrocardiographic studies revealed that ten minutes after the administration of cocaine, the heart rate remained very much the same as

The reduction produced by the prior administration of cocaine is not great.

5. The increase in cardiac output induced by ephedrine is due mainly to the increase stroke volume rather than to an increase in heart rate.

6. Throughout these studies it has been clearly demonstrated that the effects of ephedrine are lessened by the presence of cocaine. Exactly how this effect is accomplished has not been ascertained.

REFERENCES

- (1) Drill, V. A., "Pharmacology in Medicine," McGraw-Hill Book Co., Inc., New York, 1954, p. 26/15.
- (2) Chen, K. K., *J. Pharmacol. Exptl. Therap.*, 33, 219 (1928).
- (3) Tainter, M. L., *ibid.*, 36, 569 (1929).
- (4) Holck, H. G. O., *Prac. Soc. Exptl. Biol. Med.*, 30, 1087 (1933).
- (5) Shmoy, I., *Folia Pharmacol. Japan*, 49, 210 (1953).
- (6) Langendorff, O., *Arch. ges. Physiol.*, 61, 291 (1895).
- (7) Anderson, F. F., and Craver, B. N., *J. Pharmacol. Exptl. Therap.*, 93, 135 (1948).
- (8) MacIntyre, W. J., Pritchard, W. H., Eckstein, R. W., and Fridell, H. L., *Circulation*, 4, 552 (1951).
- (9) Hamet, R., *Archiv für experimentelle Pathol. Pharmacol.* 160, 1 (1931).

Anticonvulsant Activity of Pyrazole and of Pyrazole with Aliphatic Substitutions at the Three Position*

By JOHN E. OWEN, Jr.,† EDWARD E. SWANSON,‡ and DONALD B. MEYERS§

Pyrazole and a series of 3-*n*-aliphatic substituted pyrazoles have been shown to have anticonvulsant activity in rats and mice. In this study, extension of the length of the aliphatic chain up to four carbons increased the acute toxicity and anticonvulsant activity of these compounds. Further lengthening of the substituents decreased the toxicity and activity. The compounds, as anticonvulsants, were found to be more active in rats than in mice.

AMONG A SERIES of nitrogen-containing five-membered heterocycles investigated for central nervous system activity by Gibson, *et al.* (1), some substituted pyrazoles were found to protect rats from convulsions induced by electroshock and pentylenetetrazol (Metrazol). This finding has added another type of compound to the group of drugs classified as anticonvulsants. A survey of the literature has revealed relatively little information on the pharmacology of pyrazole compounds. Those on which data are available have been reported to exert a variety of actions, including central nervous system stimulation and depression, diuresis, and some musculotropic action on smooth muscle. The depression has been characterized by varying degrees of sedation, analgesia, and antipyresis. However, Gibson, *et al.* (1), appear to have been the first to report on the anticonvulsant activity of pyrazoles.

Because certain of these pyrazoles with substitutions at the 3-position were found to possess significant anticonvulsant activity, further study of a homologous series substituted at that position seemed indicated. The investigation reported here was limited to an examination of the influence of straight-chain aliphatic substitutions at the 3-position on the acute toxicity and anticonvulsant properties of pyrazole.

EXPERIMENTAL

Materials and Preparation.—The compounds

* Received May 3, 1957, from Butler University and The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

Presented to the Scientific Section, A. Ph. A., New York meeting, April-May, 1957.

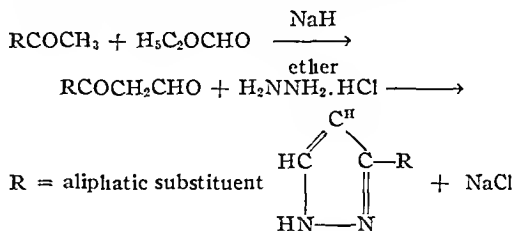
Abstracted from a thesis submitted to the Graduate School of Butler University by John E. Owen, Jr., in partial fulfillment of the requirements for the degree of Master of Science.

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studied were: pyrazole, 3-methylpyrazole, 3-n-propylpyrazole, 3-*n*-butylpyrazole, 3-*n*-amylpyrazole, and 3-*n*-nonylpyrazole. The compounds were prepared by the following general reaction:¹



These compounds, with the exception of pyrazole, are clear, yellow oils. Pyrazole is a white crystalline substance which melts at 70°. All compounds were relatively insoluble in water.

Methods.—The compounds were administered orally as gum acacia emulsions. Rats of both sexes of a Wistar derived strain, weighing 90–140 Gm.; and mice of both sexes of the Bittner-A strain, weighing 15–30 Gm., were employed for the assays.

Acute Toxicity.—The median lethal dose (LD₅₀) with standard error (S. E.) was determined for each compound in both the rats and the mice. The animals were observed for lethal effects over a period of seventy-two hours.

Anticonvulsant Activity.—Anticonvulsant activity was evaluated by the maximal electroshock seizure test (M. E. S.) of Toman, *et al.* (2), and by the subcutaneous Metrazol seizure test described by Swinyard (3). A dose response curve of anticonvulsant activity at a predetermined time of peak activity was established for each compound on each of the procedures. The median lethal and median effective (ED₅₀) doses with standard errors were computed by the Bliss method (4).

RESULTS

The data obtained from the assays of these pyrazole compounds have shown that pyrazole itself has anticonvulsant activity in rats and mice and that normal aliphatic substitutions at the 3-position quantitatively altered this activity (Tables I, II, and III).

Lengthening of the chain up to four carbon atoms increased both toxicity and anticonvulsant activity. When the chains were further increased in length to the amyl and nonyl compounds, the activity and toxicity decreased. There was one exception: 3-methylpyrazole was less active than pyrazole in rats on the subcutaneous Metrazol[®] test. A comparison of the therapeutic indexes (T. I.), calculated as LD₅₀/ED₅₀, of the three most potent of the compounds on each type of assay in both species, showed

¹ The compounds used in this study were prepared by Dr. Nelson R. Easton of the Organic Chemistry Division of The Lilly Research Laboratories.

the 3-*n*-butylpyrazole to have the optimal activity and safety margin on all procedures except the subcutaneous Metrazol test in mice. The 3-*n*-butyl compound appeared to be the most active member of the series but the 3-*n*-propylpyrazole had a slightly better T. I.

At the doses used to determine acute toxicities, the pattern of depression produced in the rats and the mice was the same for all of the compounds. The animals became ataxic with general muscle weakness followed by loss of righting reflexes and slowed respirations. They became comatose with pronounced cyanosis and death resulted from respiratory failure.

The onset of peak activity (Table IV) was more rapid and the duration of peak activity shorter in

mice than in rats. However, in rats, it was observed that at the end of peak activity time, the activity fell off very rapidly with all of the compounds except the 3-*n*-nonylpyrazole. In mice, for the most part, there was a gradual decline in the activity after the end of peak action. In Table IV the duration of activity was estimated as that interval of time, including the peak time duration, at which no less than 25% of the animals were protected from convulsions.

All compounds proved more active as anticonvulsants in rats than in mice. The 3-*n*-propylpyrazole, and 3-*n*-amylpyrazole were more toxic in rats than in mice. The T. I.'s were shown to be more favorable in rats than in mice.

DISCUSSION

The compounds were tested in both rats and mice to determine whether a species difference existed. Swinyard, *et al.*, (5), considered that either animal is suitable for this type of testing but that it is desirable for a more definitive comparison to use both species. Furthermore, unless the compounds being tested are chemically related to agents known to have antiepileptic effects, these assays may be seriously limited in predicting any clinical efficacy unless at least two species are used. In this evaluation of 3-*n*-aliphatic substituted pyrazoles as anticonvulsants, the qualitative results were similar for

TABLE I.— $LD_{50} \pm$ S. E. OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES IN RATS AND MICE

Substituent	$LD_{50} \pm$ S. E., mg/Kg	
	Rats	Mice
Hydrogen	1734.0 \pm 109.2	1456.0 \pm 96.1
Methyl	1312.0 \pm 78.7	885.5 \pm 59.3
Propyl	615.5 \pm 52.3	778.0 \pm 38.1
Butyl	509.3 \pm 25.5	543.7 \pm 24.5
Amyl	522.9 \pm 41.3	637.7 \pm 27.4
Nonyl	2695.0 \pm 207.5	1078.0 \pm 87.3

TABLE II.— $ED_{50} \pm$ S. E. AND T. I. OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES AGAINST M.E.S.

Substituent	Rats		Mice	
	$ED_{50} \pm$ S. E.	T. I.	$ED_{50} \pm$ S. E.	T. I.
Hydrogen	89.32 \pm 8.22	19.48	284.10 \pm 11.60	5.13
Methyl	66.99 \pm 6.79	19.88	173.90 \pm 6.80	5.12
Propyl	32.86 \pm 3.00	19.22	101.50 \pm 4.50	7.70
Butyl	14.70 \pm 2.06	36.35	66.99 \pm 4.42	8.23
Amyl	37.33 \pm 3.88	14.11	79.49 \pm 5.17	8.06
Nonyl	48.09 \pm 4.81	56.15	86.24 \pm 8.50	12.53

TABLE III.— $ED_{50} \pm$ S. E. AND T. I. OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES AGAINST METRAZOL INDUCED SEIZURES

Substituent	Rats		Mice	
	$ED_{50} \pm$ S. E.	T. I.	$ED_{50} \pm$ S. E.	T. I.
Hydrogen	98.47 \pm 11.32	17.70	377.6 \pm 19.2	3.89
Methyl	131.80 \pm 9.70	10.84	236.2 \pm 27.6	3.75
Propyl	76.58 \pm 13.02	8.09	138.7 \pm 8.6	5.64
Butyl	38.10 \pm 6.48	13.40	109.5 \pm 7.7	4.98
Amyl	71.93 \pm 7.34	7.35	226.0 \pm 22.7	2.82
Nonyl	141.90 \pm 20.60	19.11	327.3 \pm 17.3	3.30

TABLE IV.—PEAK TIME AND DURATION OF ANTICONVULSANT ACTIVITY OF PYRAZOLE AND 3-ALIPHATIC SUBSTITUTED PYRAZOLES

Substituent	Rats			Mice		
	Dose, mg/Kg.	Peak Time, min.	Duration, min.	Dose, mg/Kg.	Peak Time, min.	Duration, min.
Hydrogen	70	300-360	"	160	240	"
Methyl	80	300-360	"	250	90	300
Propyl	40	60-120	"	125	15-30	200
Butyl	25	30-120	"	80	15-45	75
Amyl	40	30-120	"	80	15-60	"
Nonyl	50	60-120	180	100	15-45	90

" The drug activity at onset of action rose very rapidly to the peak and fell off rapidly after peak time

both species. A quantitative difference was observed, however, since the compounds were more active in rats than in mice.

The two tests used in this investigation readily lend themselves to the evaluation of a large number of compounds (6), the usually given end points of the tests are well defined and the results are reproducible. Drugs found to be clinically effective against grand mal epilepsy will as a rule appear active in the maximal electroshock seizure test. Those agents which are active against petit mal seizures will protect animals given Metrazol subcutaneously in threshold convulsive doses. Some drugs, such as the barbiturate antiepileptics, will show a good cross activity between the two procedures and this has been confirmed clinically.

A new group of compounds with anticonvulsant activity in rats and mice has been presented. This group represents another departure from the earlier suggestions that the more potent anticonvulsant agents have certain basic similarities in the structural nuclei (7, 8). The investigation reported here has shown 3-*n*-aliphatic substituted pyrazole compounds active at dose ranges comparable to the activity found in experimental animals with some of the commonly used antiepileptic drugs. In spite of the short duration of activity in experimental animals, the potency and T I's of some of these pyrazoles suggest their suitability for clinical investigation. In view of these findings, further stud-

ies of other substitutions on pyrazole should prove fruitful in revealing more interesting compounds with neuropharmacological activity.

SUMMARY

1. Pyrazole and a series of 3-*n*-aliphatic substituted pyrazoles have been shown to possess anticonvulsant activity.

2. The optimal length of the aliphatic substitution for maximum anticonvulsant activity in both rats and mice appears to be a chain of four carbons.

3. The compounds were more active in rats than in mice as anticonvulsants.

REFERENCES

- (1) Gibson, W. R., Easton, N. R., and Swanson, E. E., to be published.
- (2) Toman, J. E. P., Swinyard, E. A., and Goodman, L. S. *J. Neurophysiol.*, **9**, 231 (1946).
- (3) Swinyard, E. A., *THIS JOURNAL*, **38**, 201 (1949).
- (4) Bliss, C. I., *Quart. J. Pharm. and Pharmacol.*, **11**, 19 (1938).
- (5) Swinyard, E. A., Brown, W. C., and Goodman, L. S. *J. Pharmacol. Exptl. Therap.*, **106**, 319 (1952).
- (6) Chen, G., Bohner, B., and Ensor, C. R., *Proc. Soc. Exptl. Biol. Med.*, **87**, 334 (1954).
- (7) Toman, J. E. P., and Goodman, L. S., *Physiol. Rev.*, **28**, 409 (1948).
- (8) Toman, J. E. P., and Taylor, J. D., *Epilepsia, Ther. Series*, **1**, 31 (1952).

Nitro-organophosphorus Compounds I.*

Phosphite and Phosphate Esters of 2-Nitro-2-methylpropanol-1

By WILLIAM SHELVER, M. I. BLAKE, and C. E. MILLER

A laboratory method has been developed for the preparation of the phosphite and phosphate esters of 2-nitro-2-methylpropanol-1. Permanganate oxidation of the phosphite yielded the phosphate.

PREVIOUS PUBLICATIONS (1-3) from this laboratory have reported the preparation of various nitroalkyl compounds for screening purposes in

connection with our cancer research program. A further pharmacological study of one of these compounds, bis(2-nitro-2-methylpropyl)-sulfite has been reported (4). Within recent months certain phosphorus derivatives such as triethylene phosphoramidate (5), have been reported as showing efficacy against certain neuroblastomas and malignant melanomas. In view of the activity of this phosphorus compound it was believed worthwhile to extend the study of aliphatic nitro-compounds to include the phosphite and phosphate esters. Such nitro alkyl esters would be

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Acknowledgment is made to the North Dakota Cancer Society for financial assistance received.

of definite value for cellular oxidation-reduction studies.

Kosolapoff (6) in his review of the chemistry of organophosphorus compounds makes no mention of the nitro alkyl esters. We wish to report our method of preparation of the hitherto unknown tris(2-nitro-2-methylpropyl)-phosphite and our method of preparation of the previously reported (7, 8) tris(2-nitro-2-methylpropyl)-phosphate.

EXPERIMENTAL

Tris(2-nitro-2-methylpropyl)-phosphite.—In a 1,000-ml. two-necked flask equipped with a dropping funnel, magnetic stirrer, and a reflux condenser, were placed 40 Gm. (0.34 mole) of 2-nitro-2-methylpropanol-1¹ dissolved in 200 ml. of dry ether and 24

portions of acetone and the acetone removed under vacuum. The resulting white solid was dissolved in a minimum quantity of acetone and precipitated by adding the acetone solution slowly to an excess of cold water. Recrystallization from the same solvent mixture gave 19 Gm. (50%) of white crystals, insoluble in water, soluble in acetone, ether, absolute alcohol, and melting at 91–93°, uncorrected. The ultraviolet spectrum (Fig. 1) was measured showing a maximum at 276 millimicrons. The molar extinction coefficient was found to be 57.92. A sample dried under high vacuum for eight hours showed the following analysis:

*Anal.*²—Calcd. for $C_{12}H_{24}N_3O_9P$: C, 37.40; H, 6.28; N, 10.90; P, 8.04. Found: C, 37.26, 37.34; H, 6.22, 6.28; N, 10.32, 10.49; P, 8.10, 8.17. The phosphite ester was characterized by oxidation to the phosphate derivative using the method of Kessler (3). The resulting crystals, after drying under high vacuum melted at 151.5–152.5°.

Tris(2-nitro-2-methylpropyl)-phosphate.—This compound was prepared in a manner analogous to that described for the phosphite, using redistilled phosphorus oxychloride. After the reaction mixture was worked up in the usual manner, there remained 20 Gm. (50%) of white crystals. The product was insoluble in water, soluble in acetone, absolute alcohol, and melted at 151.5–152.5°, uncorrected. The ultraviolet spectrum (Fig. 1) was measured showing a maximum at 278 millimicrons. The molar extinction coefficient was found to be 65.20. A sample dried under high vacuum for eight hours was used for analysis.

Anal.—Calcd. for $C_{12}H_{24}N_3O_{10}P$: P, 7.72. Found: P, 7.68. A mixed melting point of the permanganate oxidation product of the phosphite and the phosphate prepared as described showed no depression of the melting point.

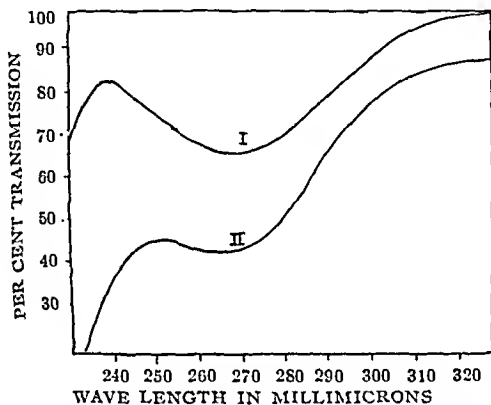


Fig. 1—Ultraviolet spectrum in absolute methanol of phosphate ester 0.002667 mole per liter (I), and phosphite ester 0.006072 mole per liter (II).

Gm. (0.30 mole) pyridine. The flask was cooled in an ice bath and 13.8 Gm. (0.1 mole) of phosphorus trichloride diluted with 100 ml. of dry ether was added over a period of three hours with stirring. The resulting mixture was filtered and the filtrate evaporated to dryness under vacuum.

The solid residue was extracted with three 200-ml.

¹ Generously supplied by Commercial Solvents Corp.

REFERENCES

- (1) Miller, C. E., and Knevel, A., *This Journal*, 43, 15 (1954).
- (2) Miller, C. E., and Krook, D., *ibid.*, 44, 99 (1955).
- (3) Kessler, W., Blake, M. I., and Miller, C. E., *ibid.*, 45, 570 (1956).
- (4) Reported New York meeting, A. Ph. A., April, 1957.
- (5) Crossley, M. L., *Cancer Research*, 12, 256 (1952).
- (6) Kosolapoff, G. M., "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950.
- (7) Vanderbilt, B. M., U. S. Pat. 2,177,757, May 19, 1938.
- (8) West, M. J., U. S. pat. 2,597,766, Sept. 29, 1950.

Analyses by Clark Micro-Phosphorus determination method.

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REFERENCES

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- (4) Bliss, C I., *Quart. J. Pharm. and Pharmacol.*, **11**, 192 (1938).
- (5) Swinyard, E A., Brown, W. C., and Goodman, L S., *J. Pharmacol. Exptl. Therap.*, **106**, 319(1952).
- (6) Chen, G., Bohner, B., and Ensor, C R., *Proc. Soc. Exptl. Biol. Med.*, **87**, 334(1954).
- (7) Toman, J E P., and Goodman, L S., *Physiol. Rev.*, **28**, 409(1948).
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A laboratory method has been developed for the preparation of the phosphite and phosphate esters of 2-nitro-2-methylpropanol-1. Permanganate oxidation of the phosphite yielded the phosphate.

PREVIOUS PUBLICATIONS (1-3) from this laboratory have reported the preparation of various nitroalkyl compounds for screening purposes in

connection with our cancer research program. A further pharmacological study of one of these compounds, bis(2-nitro-2-methylpropyl)-sulfite has been reported (4). Within recent months certain phosphorus derivatives such as triethylene phosphoramidate (5), have been reported as showing efficacy against certain neuroblastomas and malignant melanomas. In view of the activity of this phosphorus compound it was believed worthwhile to extend the study of aliphatic nitro-compounds to include the phosphite and phosphate esters. Such nitro alkyl esters would be

* Received May 3, 1957, from the School of Pharmacy, North Dakota Agricultural College, Fargo.
Presented to the Scientific Section, A PHA, New York meeting, April, 1957.

Acknowledgment is made to the North Dakota Cancer Society for financial assistance received.

of definite value for cellular oxidation-reduction studies.

Kosolapoff (6) in his review of the chemistry of organophosphorus compounds makes no mention of the nitro alkyl esters. We wish to report our method of preparation of the hitherto unknown tris(2-nitro-2-methylpropyl)-phosphite and our method of preparation of the previously reported (7, 8) tris(2-nitro-2-methylpropyl)-phosphate.

EXPERIMENTAL

Tris(2-nitro-2-methylpropyl)-phosphite.—In a 1,000-ml. two-necked flask equipped with a dropping funnel, magnetic stirrer, and a reflux condenser, were placed 40 Gm. (0.34 mole) of 2-nitro-2-methylpropanol-1¹ dissolved in 200 ml. of dry ether and 24

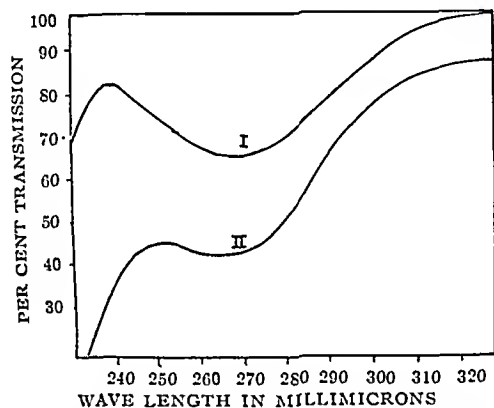


Fig. 1—Ultraviolet spectrum in absolute methanol of phosphate ester 0.002667 mole per liter (I), and phosphite ester 0.006072 mole per liter (II).

Gm. (0.30 mole) pyridine. The flask was cooled in an ice bath and 13.8 Gm. (0.1 mole) of phosphorus trichloride diluted with 100 ml. of dry ether was added over a period of three hours with stirring. The resulting mixture was filtered and the filtrate evaporated to dryness under vacuum.

The solid residue was extracted with three 200-ml.

¹ Generously supplied by Commercial Solvents Corp.

portions of acetone and the acetone removed under vacuum. The resulting white solid was dissolved in a minimum quantity of acetone and precipitated by adding the acetone solution slowly to an excess of cold water. Recrystallization from the same solvent mixture gave 19 Gm. (50%) of white crystals, insoluble in water, soluble in acetone, ether, absolute alcohol, and melting at 91–93°, uncorrected. The ultraviolet spectrum (Fig. 1) was measured showing a maximum at 276 millimicrons. The molar extinction coefficient was found to be 57.92. A sample dried under high vacuum for eight hours showed the following analysis:

*Anal.*²—Calcd. for $C_{12}H_{24}N_3O_9P$: C, 37.40; H, 6.28; N, 10.90; P, 8.04. Found: C, 37.26, 37.34; H, 6.22, 6.28; N, 10.32, 10.49; P, 8.10, 8.17. The phosphite ester was characterized by oxidation to the phosphate derivative using the method of Kessler (3). The resulting crystals, after drying under high vacuum melted at 151.5–152.5°.

Tris(2-nitro-2-methylpropyl)-phosphate.—This compound was prepared in a manner analogous to that described for the phosphite, using redistilled phosphorus oxychloride. After the reaction mixture was worked up in the usual manner, there remained 20 Gm. (50%) of white crystals. The product was insoluble in water, soluble in acetone, absolute alcohol, and melted at 151.5–152.5°, uncorrected. The ultraviolet spectrum (Fig. 1) was measured showing a maximum at 278 millimicrons. The molar extinction coefficient was found to be 65.20. A sample dried under high vacuum for eight hours was used for analysis.

Anal.—Calcd. for $C_{12}H_{24}N_3O_{10}P$: P, 7.72. Found: P, 7.68. A mixed melting point of the permanganate oxidation product of the phosphite and the phosphate prepared as described showed no depression of the melting point.

REFERENCES

- (1) Miller, C. E., and Knevel, A., *This Journal*, **43**, 15 (1954).
- (2) Miller, C. E., and Krook, D., *ibid.*, **44**, 99 (1955).
- (3) Kessler, W., Blake, M. I., and Miller, C. E., *ibid.*, **45**, 570 (1956).
- (4) Reported New York meeting, A. Ph. A., April, 1957.
- (5) Crossley, M. L., *Cancer Research*, **12**, 256 (1952).
- (6) Kosolapoff, G. M., "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950.
- (7) Vanderbilt, B. M., U. S. Pat. 2,177,757, May 19, 1938.
- (8) West, M. J., U. S. pat. 2,597,766, Sept. 29, 1950.

² Carbon, hydrogen, and nitrogen analyses by Clark Micro-analytical Laboratory, Urbana, Ill. Phosphorus determination by Parr Bomb-Gravimetric method.

Book Notices

The Neurohypophysis. Edited by H. HELLER. Academic Press Inc., New York, 1957. xv + 275 pp. 19 x 25.5 cm. Price \$9.50.

This compilation of the papers and the discussions at the eighth symposium of the Colston Research Society held in 1956 develops the two main lines of progress in the study of the neurohypophysis: the evidence that the neurohypophyseal hormones are elaborated not in the posterior pituitary lobe, but in the hypothalamus; and the establishment of the chemical nature of the active principles—in some mammalian species. The titles of the papers are: Evidence concerning the endocrine function of the neurohypophysis and its nervous control; Relationship between neurohypophyseal structure and function; The comparative aspect of neurosecretion with special reference to the hypothalamo-hypophyseal system; The relationship of oxytocin and vasopressin to active proteins of posterior pituitary origin. Studies concerning the existence or non-existence of a single neurohypophyseal hormone; Polypeptides with posterior pituitary-like activities; The storage and liberation of neurohypophyseal hormones, The metabolism and fate of neurohypophyseal hormones, The excretion of posterior pituitary principles in the urine; The effects of hemorrhage and plasma hypertonicity on the neurohypophysis; Polydipsia, antidiuresis and milk ejection caused by hypothalamic stimulation; Conditions under which posterior pituitary hormones increase sodium and potassium excretion by the kidney; The location of antidiuretic action in the mammalian kidney; The antidiuretic action of neurohypophyseal hormones in Amphibia; Neurohypophyseal hormones and the mammary gland; On oxytocin and uterine function; The release of vasopressin and oxytocin in response to drugs; The adrenal-neurohypophyseal relationship; and Comparative aspects of adrenocortical-neurohypophyseal relationships. References are given after each paper, but it is too bad that this beautifully printed and bound book does not include a general index as a reference aid. The book will be of particular interest to biochemists, physiologists, and pharmacologists.

Vitamin A. By THOMAS MOORE. D. Van Nostrand Company, Inc., N. J., U. S. distributors for Elsevier Publishing Company, London, 1957. xx + 645 pp. 14.5 x 22.5 cm. Price \$14.

This book is presented as a comprehensive review on vitamin A compiled from data obtained by nutritionists, biochemists, chemists, and workers in related fields. The 39 chapters are grouped under the main headings: Historical introduction, Estimation of vitamin A and its provitamins and congeners, Comparative biochemistry and natural history of the carotenoids and vitamin A, Physiology and biochemistry of vitamin A and its provitamins and congeners, Pathology of vitamin A deficiency or excess, Vitamin A in the human, and Special topics. An appendix includes vitamin A deficient diets for

animals, determination of carotene in biological materials, and estimation of vitamin A by ultraviolet spectrophotometry. References are given at the end of each chapter, and a very helpful aid in their use is the notation at the bottom of every odd numbered page as to where the next group of references will be found. An author index and a good subject index add to the value of this excellently printed book as an important reference.

Handbook of Solvents. Vol. I: Pure Hydrocarbons. By IBERT MELLAN. Reinhold Publishing Corporation, New York, 1957. iv + 249 pp. 16 x 23.5 cm. Price \$6.50.

This book attempts a systematic arrangement of the pure hydrocarbons used as solvents, grouping them according to distillation range as a means of bringing solvents with similar properties together. Solvents are listed by trade or commercial names, rather than by chemical designation. The book is divided into an introductory section in which tests are described and relevant general information is given. This is followed by tabulated specifications for aliphatic hydrocarbons, aromatic hydrocarbons, and terpenes. A subject index is appended. This excellently printed and well-bound book should be a useful reference in school and commercial laboratories.

Handbook of Material Trade Names. Supplement I to the 1953 edition. By O. T. ZIMMERMAN and IRVIN LAVINE. Industrial Research Service, Inc., Dover, N. H., 1956. x + 383 pp. 19 x 27 cm. Probable price \$12.50.

This supplement includes new items and brings up to date the status of many trade names that have been registered as trade-marks since the 1953 edition of this Handbook. The system, style, and format of the supplement are similar to the 1953 edition of the Handbook of Material Trade Names which was reviewed in *THIS JOURNAL*, 42, 385(1953). Those who have found the earlier volume useful will welcome the issuance of supplement I. Supplement II is being prepared for publication.

Selected Scientific Papers from the Institute Superiores di Sanità (Rome). Vol. 1, Part 1, 1957. Interscience Publishers, Inc., New York, 1957. 233 pp. 17 x 24 cm. Price \$8.10.

This book is a compilation of articles of general scientific interest and of particular interest in the field of public health. Subjects include: "The low energy gamma ray spectrum of cosmic radiation," and a section on the genetics and control of insects, particularly the house-fly. Of particular interest to pharmacists is the article on "Chemical and pharmacological studies of the alkaloids of *Strychnos* sp. from Brazil." This paper-back book is beautifully printed and illustrated, but it does not have a general index.

Solvent Extraction in Analytical Chemistry. By GEORGE H. MORRISON and HENRY FREISER. John Wiley & Sons, Inc., New York, 1957. xi + 269 pp. 15 x 23 cm. Price \$6.75.

This book covers the theory and practice of solvent extraction methods that are applicable to rapid analyses for trace concentrations and highly complex mixtures of inorganic compounds and materials. The first part of the book takes up Principles of solvent extraction, gives a classification of metal extraction systems and includes chapters on: Formation of metal complexes; Distribution of the extractable species; Chemical interaction in the organic phase; Quantitative treatment of extraction equilibria; and Kinetic factors in extraction. Part 2 includes methods and techniques of extraction. Part 3 covers ion association and chelation in extraction systems, and Part 4 includes selected procedures for the extraction of the elements. A tabulation of organic solvents and their physical constants is appended.

The pharmaceutical analyst can note with relief the statement "Fortunately, emulsion problems are not often encountered in most extraction separations employed for analytical purposes" (in the inorganic field). The sound observation is made that "The method of agitation undoubtedly influences the particle size, and analytical extractions involving solvents with a tendency to form emulsions are best performed with a continuous type of extractor."

The text is thoroughly documented with references including recent reports. An index lists the elements and the reagents used in their extraction. A subject index is appended. The book has a good format, clear type and figures, and a good binding. It is a very useful addition to the technical library and should be helpful to teachers and practicing analysts.

Rendiconti Istituto Superiore di Sanità. Vol. XVII, 1954. English Edition of Special Number on Pilot Plant Techniques of Submerged Fermentation. Edited by DOMENICO MAROTTA and E. B. CHAIN. Published under the auspices WHO, 1957, copyright by Fondazione Emanuele Paternò, Rome, 1954. x + 243 pp. 17 x 24 cm. Price: Lit. 5,000.

The text contains twelve chapters entitled: Aeration studies; A laboratory fermenter for vortex and sparger aeration; Fermenters of 90 and 300 L. capacity for vortex and sparger aeration; A "compensated" stuffing box and bearing unit for fermenters of semi-industrial and industrial capacities; Pilot plant for fermentation in submerged culture; A simple rotary shaker; Antifoam agents in aerobic fermentation; Evaluation of the activities of antifoam preparations; Inter-relation of protein and polynucleotide synthesis in *Escherichia coli*; The effect of mechanical agitation on the morphology of *Penicillium chrysogenum* Thom in stirred fermenters; *Romanoa*, a new genus of soil fungus with antibacterial activity; Genetics of *Penicillium chrysogenum*. I Heterokaryosis in *Penicillium chrysogenum*; and Genetics of *Penicillium chrysogenum*. II Segregation and recombination from a heterozygous diploid. Each section begins with a summary of the part of the text (in English,

Italian, French, and German versions). The text is in English, with good type and excellent illustrations, but with a paper-back binding. The subject index is hardly more useful than the table of contents for reference purposes, but references are given at the end of each chapter.

Ion Exchangers in Organic and Biochemistry. Edited by CALVIN CALMON and T. R. E. KRESSMAN, Interscience Publishers, New York, 1957. xii + 761 pp. 15 x 23 cm. Price \$15.

The editors of this book have attempted to encompass the great increase in knowledge in the field of ion exchange by having various phases of the field covered by 37 authoritative contributors. The book is divided into three parts: the first two dealing with fundamentals and the techniques and apparatus used in the exchange and separation of ions, while the third part deals with applications to specific aspects of organic and biochemistry. Part III covers first, the ion exchange characteristic of body tissue and cells, followed by: organisms, protein materials, special body products and fluids, pharmaceutical and medical applications, plants and plant products, foods, organic chemistry, catalysis, and water.

Particularly interesting to the pharmacy field are the chapters on: Medical and pharmaceutical applications, Isolation and purification of antibiotics, Vitamins, Alkaloids, Nonsteroid hormones, Isolation and analysis of the components of urine, Sodium and potassium removal *in vivo*, and Gastric acidity. The style, type, format, and binding of the book are very good. Each chapter has its references, and author and subject indexes are appended. This book is an excellent addition to the modern scientific library.

Scoville's The Art of Compounding. 9th ed. By GLENN L. JENKINS, DON E. FRANCKE, EDWARD A. BRECHT, and GLEN J. SPERANDIO. The Blakiston Division, McGraw-Hill Book Company, Inc., New York, 1957. vii + 551 pp. 18 x 25 cm. Price \$11.

This excellent book, which is devoted to a clear and comprehensive treatment of pharmaceutical compounding and dispensing, is useful as a textbook for students and as a reference book for practitioners. The authors have avoided the inclusion of material that is readily available in other books and compendia that should be conveniently at hand. This permits full treatment of the selected material without the need for handling an oversize book.

The ninth edition is similar to the eighth edition, which was reviewed in THIS JOURNAL, 40, 362 (1951), with respect to its fine style and format, its clear type and illustrations, and the clarity of its text. A new chapter on ophthalmic solutions has been included and much new material has been added to give the text the new look in modern compounding techniques and formulations. The thorough treatment of incompatibilities is retained as one of the valuable aids for study and reference afforded by this very useful book. It can be recommended without reservation.

British National Formulary (Standard Edition)

Edited by the Joint Formulary Committee of the British Medical Association and The Pharmaceutical Society of Great Britain The Pharmaceutical Press, London, 1957 226 pp 10 5 x 16 5 cm Price 6s 6d, Interleaved copies 10s

This formulary for prescribers in hospital and general practice is intended also to be useful to pharmacists in Great Britain, particularly as these practices and practitioners are related to the British National Health Service However, the Joint Formulary Committee notes "that a doctor is free to prescribe what he considers to be in the best interests of his patient, *whether or not the preparation appears in the British National Formulary*"

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Foundation Symposium Edited by G E W WOLSTENHOLME and CECILIA M O'CONNOR Little, Brown and Company, Boston, 1957 xi + 327 pp 13 5 x 20 5 cm Price \$9

This compilation of papers and discussions includes the following reports Synthesis and properties of purines of potential biological interest, Some synthetic studies on purines and related heterocycles, Some new *N* methylpurines, The structure of the hydroxypurines investigated by *O* and *N*-methylation, The spectra and structure of the monohydroxypurines and other potentially tautomeric purines, the π -electron properties of purine calculated by the L C A O method, The degradation of uric acid by water under pressure, The 8 position in purines The chemical and biological transformation of purines into pteridines, *Cyclonucleosides*, Stereochemistry of nucleoside synthesis, Synthetic chemical investigations related to the metabolism of purines, The effects of potential antipurines on a purine requiring strain of *Escherichia coli*, The

chemistry of new purines in the B₁₂ series of vitamins, Biological and microbiological activity of purine analogues of vitamin B₁₂, Puromycin, Chemical and biological behaviors of 9- β -D-ribofuranosylpurine, On the activation of the one carbon unit for the biosynthesis of purine nucleotides, The enzymatic synthesis of inosinic acid *de novo*, Enzymic control of purines by xanthine oxidase, The biological effects of 8 azapurines, Biochemical effects of 6 mercaptopurine, and The use of 6-mercaptopurine in the treatment of leukemia References are given after each paper and a subject index is appended The book should be available to graduate students in the related sciences and should be of particular interest to advanced workers in chemistry and biology

The Leukemias Etiology, Pathophysiology, and

Treatment Edited by JOHN W REBUCK, FRANK H BETHELL, and RAYMOND W MONTO Academic Press Inc, New York, 1957 xi + 711 pp 15 x 23 cm Price \$13

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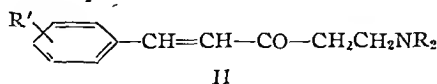
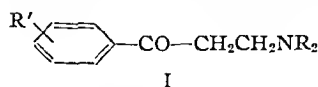
NUMBER 2

Ketonic Mannich Bases and the Products of their Reduction and Bromination*

By W. LEWIS NOBLES† and J. H. BURCKHALTER

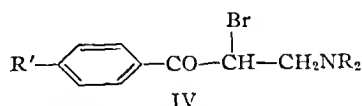
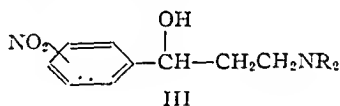
A group of ketonic bases, indicated as types I and II, were synthesized by means of the Mannich reaction for pharmacological study. Certain of the type I Mannich bases were then reduced by means of aluminum isopropoxide to nitrophenyl amino alcohols (type III) for use as side chains in future studies. Other type I bases were brominated to give ketonic nitrogen mustards for study against cancer. Except for appreciable *in vitro* antituberculous activity of some of the Mannich bases, there were no noteworthy pharmacological results.

INTEREST HAS BEEN EXPRESSED in the pharmacological activity of various ketonic Mannich bases (1). Therefore, we wished to prepare a number of unavailable Mannich bases of types I and II and to submit them for pharmacological

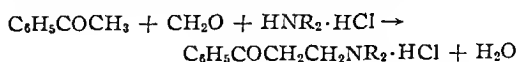


testing. Another important planned phase of these studies involved the reduction of type I, where R' is nitro, to compounds of type III which

would be employed as inter mediates in the synthesis of potential amebacides, as described in the next paper which appears in THIS JOURNAL. Further, it was planned to obtain a group of ketonic nitrogen mustards of type IV as possible anticancer agents.



The preparation of compounds of types I and II, which are listed in Tables I and II, respectively, was carried out by means of the Mannich reaction (2). It involved the interaction of formaldehyde or paraformaldehyde with the appropriate ketone and amine hydrochloride, illustrated as follows:



In general, the reactions proceeded smoothly and in good yields to give the expected product. The fact that excellent yields are seldom obtained in the Mannich reaction involving ketones is understandable when it is noted that there is more than one replaceable hydrogen in the ketone ordinarily employed, which leads to by-product formation. Also, inconsistent results in our

* Received July 26, 1957, from the Laboratory of Pharmaceutical Chemistry, University of Kansas School of Pharmacy, Lawrence, Kan.

Extracted from a portion of the Ph. D. thesis of W. Lewis Nobles, 1952. For the first paper extracted from this thesis, see THIS JOURNAL, 42, 176(1953).

† Fellow of the American Foundation of Pharmaceutical Education. Present address: School of Pharmacy, University of Mississippi, University, Miss.

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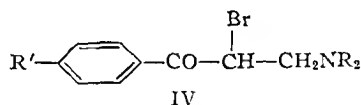
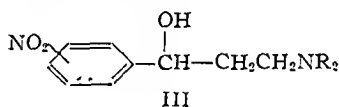
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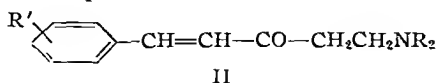
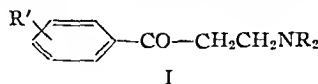
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A group of ketonic bases, indicated as types I and II, were synthesized by means of the Mannich reaction for pharmacological study. Certain of the type I Mannich bases were then reduced by means of aluminum isopropoxide to nitrophenyl amino alcohols (type III) for use as side chains in future studies. Other type I bases were brominated to give ketonic nitrogen mustards for study against cancer. Except for appreciable *in vitro* antituberculous activity of some of the Mannich bases, there were no noteworthy pharmacological results.

would be employed as inter mediates in the synthesis of potential amebicides, as described in the next paper which appears in THIS JOURNAL. Further, it was planned to obtain a group of ketonic nitrogen mustards of type IV as possible anticancer agents.

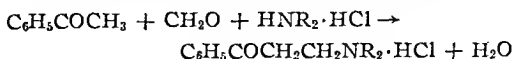


INTEREST HAS BEEN EXPRESSED in the pharmacological activity of various ketonic Mannich bases (1). Therefore, we wished to prepare a number of unavailable Mannich bases of types I and II and to submit them for pharmacological



testing. Another important planned phase of these studies involved the reduction of type I, where R' is nitro, to compounds of type III which

The preparation of compounds of types I and II, which are listed in Tables I and II, respectively, was carried out by means of the Mannich reaction (2). It involved the interaction of formaldehyde or paraformaldehyde with the appropriate ketone and amine hydrochloride, illustrated as follows:

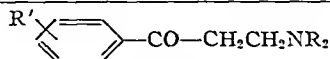


In general, the reactions proceeded smoothly and in good yields to give the expected product. The fact that excellent yields are seldom obtained in the Mannich reaction involving ketones is understandable when it is noted that there is more than one replaceable hydrogen in the ketone ordinarily employed, which leads to by-product formation. Also, inconsistent results in our

* Received July 26, 1957, from the Laboratory of Pharmaceutical Chemistry, University of Kansas School of Pharmacy, Lawrence, Kan.

† Extracted from a portion of the Ph. D. thesis of W. Lewis Nobles, 1952. For the first paper extracted from this thesis, see THIS JOURNAL, 42, 176(1953).

‡ Fellow of the American Foundation of Pharmaceutical Education. Present address: School of Pharmacy, University of Mississippi, University, Miss.

TABLE I.— β -DIALKYLAMINOPROPIONPHENONES

No	R'	NR ₂	Yield, %	M. P., °C.	Formula	Ionic Calcd.	Halogen Found
1	<i>p</i> -Chloro	Dimethylamino	71	176	C ₁₁ H ₁₄ ClNO·HCl ^{a,b}
2	<i>p</i> -Chloro	Diethylamino	60	145	C ₁₃ H ₁₈ ClNO·HCl ^a	12.84	12.83
3	<i>p</i> -Chloro	1-Piperidyl	56	190	C ₁₄ H ₁₈ ClNO·HCl ^c	12.30	12.37
4	<i>p</i> -Bromo	Dimethylamino	52	196	C ₁₁ H ₁₄ BrNO·HCl ^d
5	<i>p</i> -Bromo	Diethylamino	40	166	C ₁₃ H ₁₈ BrNO·HBr	21.88	22.01
6	<i>p</i> -Bromo	1-Piperidyl	42	188	C ₁₄ H ₁₈ BrNO·HBr	21.19	21.12
7	<i>p</i> -Bromo	1-Pyrrolidyl	69	199	C ₁₃ H ₁₆ BrNO·HCl	11.13	11.29
8	<i>p</i> -Methoxy	Dimethylamino	75	181	C ₁₂ H ₁₇ NO ₂ ·HCl ^e
9	<i>p</i> -Methoxy	1-Pyrrolidyl	38	184	C ₁₄ H ₁₉ NO ₂ ·HCl	13.14	13.22
10	<i>p</i> -Methoxy	Benzylamino	29	183	C ₁₇ H ₁₉ NO ₂ ·HCl ^f
11	<i>m</i> -Methoxy ^g	Dimethylamino	81	168	C ₁₂ H ₁₇ NO ₂ ·HCl ^d
12	<i>o</i> -Hydroxy	Dimethylamino	33	156	C ₁₁ H ₁₅ NO ₂ ·HCl	15.43	15.55
13	<i>m</i> -Hydroxy ^g	Dimethylamino	50	180	C ₁₁ H ₁₅ NO ₂ ·HCl ^d
14	<i>p</i> -Hydroxy ^h	Dimethylamino	56	192	C ₁₁ H ₁₅ NO ₂ ·HCl ^d
15	<i>p</i> -Phenyl ⁱ	Dimethylamino	69	192	C ₁₇ H ₁₉ NO·HCl	12.24	12.39
16	<i>p</i> -Nitro ^m	Dimethylamino	72	191	C ₁₁ H ₁₄ N ₂ O ₃ ·HCl ^j	13.71	13.80
17	<i>p</i> -Nitro	Diethylamino	66	150	C ₁₃ H ₁₈ N ₂ O ₃ ·HCl ^k	12.36	12.22
18	<i>p</i> -Nitro	1-Pyrrolidyl	61	185	C ₁₃ H ₁₆ N ₂ O ₃ ·HCl ^h	12.45	12.60
19	<i>p</i> -Nitro	1-Piperidyl	51	200	C ₁₄ H ₁₈ N ₂ O ₃ ·HCl ^h
20	<i>p</i> -Nitro	Diethylamino	26	140	C ₁₃ H ₁₈ N ₂ O ₃ ·HCl	11.26	11.39
21	<i>p</i> -Nitro	Diethanolamino	19	146	C ₁₃ H ₁₈ N ₂ O ₅ ·HCl ^h	11.12	11.31
22	<i>p</i> -Nitro	4-Morpholinyl	62	218	C ₁₅ H ₁₆ N ₂ O ₄ ·HCl ^h	11.79	11.82
23	<i>m</i> -Nitro ⁿ	Dimethylamino	72	205	C ₁₁ H ₁₄ N ₂ O ₃ ·HCl ^l
24	<i>m</i> -Nitro	Diethylamino	..	122	C ₁₃ H ₁₈ N ₂ O ₃ ·HCl ^l
25	<i>m</i> -Nitro	1-Pyrrolidyl	63	182	C ₁₃ H ₁₆ N ₂ O ₃ ·HCl	12.45	12.54
26	<i>m</i> -Nitro	1-Piperidyl	..	180	C ₁₄ H ₁₈ N ₂ O ₃ ·HCl ^l

^a Adamsoo, D. W., and Billingham, J. W. *J. Chem. Soc.*, 1950, 1039^b Dhont, J., and Wibaut, J. P., *Rec. trav. chim.*, 63, 81(1944).^c Deaton, J. J., et al., *J. Am. Chem. Soc.*, 71, 2048(1949).^d Koott, E. H., *J. Chem. Soc.*, 1947, 1190^e Maouich, C., and Lammeriog, D., *Ber.*, 55, 3510(1922)^f Prepared by John A. Durden, This Laboratory. Anal. Calcd C, 66.76; H, 6.59. Found: C, 66.74; H, 6.38^g See ref. 5^h See ref. 5-cⁱ Maouich, C., and Daoehi, M., *Arch. Pharm.*, 276, 206(1938).^j Intermediate *m*-methoxyacetophenone made from Hilton-Davis *m*-hydroxyacetophenone, according to Auwers, K. V., et al., *Ann.*, 408, 212(1915)^k *p*-Hydroxyacetophenone obtained through the courtesy of Dow Chemical Co^l To effect the Mannich reaction, amyl alcohol was used as the solvent^m *p*-Nitroacetophenone obtained through the courtesy of Monsanto Chemical Coⁿ *m*-Nitroacetophenone obtained through the courtesy of Carbide and Carbon Chemicals Corp.

studies were sometimes encountered. For example, the first two attempts to obtain compound 15 (Table I) resulted only in the recovery of starting material. However, when the customary solvent alcohol was replaced by isoamyl alcohol, uniformly good yields were obtained. The latter alcohol, having a higher reflux temperature, presumably afforded the necessary elevation of temperature to effect reaction. Under the conditions employed, it was not possible to cause 3,5-dinitroacetophenone or *p*-nitro- α -acetamidoacetophenone, a chloramphenicol intermediate, to undergo the Mannich reaction, although the latter will easily hydroxymethylate (3).

The *p*-nitro- β -dialkylaminopropiophenones of Table I are so unstable that they decompose readily in the presence of dilute alkali. However, they can be prepared and stored for some time in the form of their hydrochloride salts. The ready decomposition of ketonic Mannich bases into α,β -unsaturated ketones and amines is

well established, although they generally are not so unstable as the ones under discussion (4).

A Mannich base from *p*-nitroacetophenone (Compound 16, Table I) apparently was first prepared in This Laboratory (5), and it was made as the parent compound of the corresponding vinyllog referred to earlier (1). The *in vitro* fungicidal activity of the former was of sufficient interest to lead to the synthesis of several compounds listed in Table I.

The availability of such compounds as *p*-nitro- β -dimethylaminopropiophenone (5) (Table I) suggested them as possible intermediates to be used as side chains in the synthesis of products of interest as amebicidal and antimalarial agent. In This Laboratory attempts were made by W. C. Ling to reduce such compounds to the corresponding 1-(*p*-aminophenyl)-3-dialkylaminopropiophenols or ketones (6). While the catalytic reduction of *m*-nitro- β -piperidylpropionphenone hydrochloride to the corresponding *m*-aminophenyl

ketone has been reported (7), the melting point and solubility of the product were found to change rapidly upon standing (6). The logical explanation of the instability of the desired compounds is the formation of Schiff bases, and thus it appeared that a means must be found of reducing type I compounds to the stable nitro amino-alcohols (III), which later could be reduced further catalytically to the desired side-chain amines

It was evident that, owing to their instability, a successful reduction of the type of Mannich base in question (I) must be applied directly to the amine salt. The Meerwein-Ponndorf-Verley method, employing aluminum isopropoxide, appeared to fulfill the requirements, for, in contrast to other reductions involving metals in acid or alkaline media or even certain catalytic hydrogenations, carbon-carbon double bonds, nitro

TABLE II.—5-DIALKYLAMINO-1-PHENYL-1-PENTEN-3-ONES

No	Phenyl Substituents	NR ₂	Yield, %	M. P., °C	Formula	Chlorine	
						Calcd	Found
1	None ^a	4-Morpholinyl	66	178	C ₁₅ H ₁₉ NO ₂ ·HCl	12 58	12 46
2	None ^a	1-Pyrrolidyl	42	178	C ₁₅ H ₁₉ NO·HCl	13 34	13 51
3	2,3-Dimethoxy ^b	1-Pyrrolidyl	65	155	C ₁₇ H ₂₃ NO ₃ ·HCl	10 88	11 01
4	<i>p</i> -Nitro ^b	1-Pyrrolidyl	57	196	C ₁₅ H ₁₃ N ₂ O ₃ ·HCl	11 41	11 53

^a Made from Fastman benzalacetone

^b For intermediate ketone, see ref 1

TABLE III.—3-DIALKYLAMINO-1-PHENYL-1-PROPANOLS

No	R,	NR ₂	Yield, %	M. P., °C	Formula	Chlorine	
						Calcd	Found
1	None	Dimethylamino	65	134	C ₁₁ H ₁₇ NO·HCl ^a		
2	<i>p</i> -Nitro	Dimethylamino	65	176	C ₁₁ H ₁₆ N ₂ O ₃ ·HCl ^b		
3	<i>p</i> -Nitro	Dimethylamino	54	140	C ₁₃ H ₂₀ N ₂ O ₃ ·HCl	12 28	12 41
4	<i>p</i> -Nitro	1-Pyrrolidyl	61	168	C ₁₅ H ₁₈ N ₂ O ₄ ·HCl	12 37	12 52
5	<i>p</i> -Nitro	1-Piperidyl	55	177	C ₁₄ H ₂₀ N ₂ O ₃ ·HCl	11 78	11 91
6	<i>p</i> -Nitro	4-Morpholinyl	67	185	C ₁₃ H ₁₈ N ₂ O ₄ ·HCl	11 71	11 66
7	<i>m</i> -Nitro	Dimethylamino	42	183	C ₁₁ H ₁₆ N ₂ O ₃ ·HCl	13 59	13 71

^a Mannich, C, and Heilner, G, *Ber.*, 55, 356(1922) made by catalytic hydrogenation using palladium. See also Experimental section of this report

^b Anal: Calcd C, 50.67, H, 6.57. Found: C, 50.89, H, 6.47

TABLE IV.—α-BROMO-β-DIALKYLAMINOPROPIOPHENONES

No	R'	NR ₂	Yield, %	M. P., °C	Formula	Total ionic halide ^d	
						Calcd	Found
1	H	1-Piperidyl	85	185	C ₁₄ H ₁₈ BrNO·HBr ^a		
2	H ^b	4-Morpholinyl	85	181	C ₁₃ H ₁₆ BrNO ₂ ·HBr	42 16	42 03
3	Cl ^c	Dimethylamino	78	191	C ₁₁ H ₁₃ BrClNO·HCl ^d	35 27	35 39
4	Cl ^c	1-Piperidyl	82	175	C ₁₄ H ₁₇ BrClNO·HBr	38 84	39 01
5	Br ^e	Diethylamino	80	150	C ₁₃ H ₁₇ Br ₂ NO·HBr	35 99	35 87
6	Br ^e	1-Piperidyl	81	168	C ₁₄ H ₁₇ Br ₂ NO·HBr	35 07	34 86
7	OCH ₃ ^f	Dimethylamino	86	169	C ₁₂ H ₁₆ BrNO ₂ ·HBr	43 54	43 39
8	OCH ₃ ^f	1-Piperidyl	87	145	C ₁₅ H ₂₀ BrNO ₂ ·HBr	39 25	39 30
9	NO ₂ ^g	1-Piperidyl	84	182	C ₁₄ H ₁₇ BrN ₂ O ₂ ·HBr	37 87	37 88

^a Ref 10

^b Intermediate Mannich base hydrobromide made from morpholine hydrobromide in 52% yield, m p 192° Not analyzed

^c See Table I, compound 1, for intermediate Mannich base hydrochloride

^d Note that the α bromo group reacts with silver nitrate in the analytical procedure, and that the analysis of compound 3 includes both bromide and chloride ions

^e Intermediate Mannich base hydrobromide made in 55% yield, m p 205° Anal: Calcd Br, 24.02 Found 23.78

^f Intermediate Mannich base hydrobromide, Table I, compound 5

^g See Table I, compound 6 for intermediate

^h Intermediate Mannich base hydrobromide made in 75% yield, m p 182°

ⁱ Intermediate Mannich base hydrobromide made in 75% yield by John A. Durden, m p 223° Ana Calcd C 54.88 6.76 Found C, 55.02; H, 6.59

^j Intermediate Mannich base hydrobromide made in 50% yield, m p 189°

groups and carboxylic esters are not reduced by this agent. This procedure had previously been applied by Lutz and associates (8) to Mannich bases derived from *p*-haloacetophenones, as well as to a number of α -amino ketones. While the reduction of the latter proceeded very smoothly, in only one case was the amino alcohol obtained, and the yield was less than 10%. These poor results were attributed to reductive fission of the dialkylamino groups (8). In the early stages of our studies, the same results were noted, for, in each case as the temperature was elevated, the evolution of the low boiling dialkylamine was detected (9). Meanwhile, the contents of the reaction vessel became dark and resinous. Apparently, as the amine was eliminated, polymerization of the residual phenyl vinyl ketone occurred.

The addition of aluminum chloride has been successfully employed by Lutz (8) to improve yields in the reduction of α -amino ketones by aluminum isopropoxide, although it apparently has not been used in the reduction of Mannich bases, which are β -amino ketones. However, we found this procedure to work well when a half mole of aluminum chloride was used for each mole of Mannich base. The compounds prepared by this method are outlined in Table III and are derived from those of Table I. However, the general procedure is illustrated in the Experimental section by application to a type II compound, which is vinylogous with type I.

A group of ketonic nitrogen mustard compounds of type IV (see Table IV) were synthesized by bromination of ketonic Mannich bases (type I) using the procedure of Land, Ziegler, and Sprague (10).

Pharmacological Results.¹—The compounds of Table I were screened generally for antibacterial and antiamebic activity. Several, such as numbers 8, 10, and 22 showed interesting *in vitro* antituberculous activity but later *in vivo* tests in mice were negative. Others, such as 1, 4, 8, 16, and 21 showed promise against histoplasmosis but again were inactive *in vivo*. Compound 1, Table II, showed *in vitro* antituberculous activity, but failed in mice. Also, its antiamebic and analgetic activity were of too low an order to be of further interest.

The products of Table III were utilized as chemical intermediates and were not tested pharmacologically, while the ketonic nitrogen mustards of Table IV were found by Dr. Chester C.

Stock of Sloan-Kettering Institute to be devoid of anticancer activity. This lack of activity is not surprising in view of the fact that known anticancer agents contain two β -haloethyl groupings, whereas ours have only one. Compound 8 did, however, show appreciable analgetic activity but was toxic to guinea pigs.

It is of passing interest to point out that the compounds of Table IV are strongly sternutatory. Their structural relationship to phenacyl chloride, an α -halo ketone and a famous tear gas, is noteworthy.

EXPERIMENTAL²

β -Dialkylaminopropiophenones (Table I).—The procedure of Maxwell (11) was employed for the synthesis of the ketonic Mannich base hydrochlorides of Table I. In general, 95% alcohol was used as the recrystallizing solvent, although compounds 1 and 2 were recrystallized from isopropyl alcohol while 3, 7, 9, 12, and 17 were purified by means of an alcohol-acetone mixture.

Most of the intermediate ketones were Eastman chemicals, otherwise the source is indicated in the footnotes to Table I.

2-(β -1-Pyrrolidylpropionyl)-thiophene Hydrochloride.—By means of the foregoing procedure, this compound was prepared in 48% yield, m. p. 157–162°. After recrystallization from alcohol-acetone, the m. p. became 169–170°.

Anal. Calcd. for $C_{11}H_{13}NOS \cdot HCl$: Cl, 14.43. Found: Cl, 14.60.

2-(β -Benzylaminopropionyl)-thiophene Hydrochloride.³—By means of the foregoing procedure, this compound was prepared in 25% yield, m. p. 172–173°. After recrystallization from absolute alcohol, the m. p. became 174–175°.

Anal. Calcd. for $C_{14}H_{15}NOS \cdot HCl$: C, 59.66; H, 5.72. Found: C, 59.36; H, 5.44.

5-Dialkylamino-1-phenyl-1-penten-3-ones (Table II).—The compounds of this table were prepared by the procedure of Table I. The products, obtained as the hydrochlorides, were recrystallized from an alcohol-acetone mixture.

5-Dimethylamino-1-(*p*-nitrophenyl)-1-penten-3-ol Hydrochloride.—To a hot slurry of 20 Gm. (0.1 mole) of aluminum isopropoxide and 3.3 Gm. (0.025 mole) of anhydrous aluminum chloride in 175 ml. of dry isopropyl alcohol contained in a 500-ml. three-necked flask equipped with a mechanical stirrer and reflux condenser, there was added 14.3 Gm. (0.05 mole) of 5-dimethylamino-1-(*p*-nitrophenyl)-1-penten-3-one hydrochloride (1). The mixture was brought to full reflux and maintained at that temperature for fifteen minutes. Then, the upright condenser was replaced by one turned downward for distillation, and stirring and removal of acetone continued until the distillate gave a negative acetone test (about two hours). The upright condenser was reinserted and full reflux maintained for about ten minutes. Once again, the upright condenser was replaced by one placed so as to allow

¹ The authors wish to thank Parke, Davis and Company for arranging for the pharmacological testing.

² Melting points are uncorrected.

³ Prepared by John A. Durden of This Laboratory.

distillation to proceed and a few drops of distillate was collected. Since the acetone test was still negative, it was concluded that reduction was complete. The residual isopropyl alcohol was removed *in vacuo*. The solid residue was cooled and then treated with 200 ml. of ice-cold 10% hydrochloric acid. This suspension was dissolved in about 375 ml. of water. With cooling and stirring, the aqueous solution was made strongly basic (pH about 12) with 40% potassium hydroxide. The basic solution was thoroughly extracted with ether, and the extract was washed with saturated sodium chloride solution and then dried with anhydrous sodium sulfate overnight. The ether extract was then filtered free of the drying agent and treated with anhydrous hydrogen chloride. An orange oil was thus produced. On standing for forty-eight hours in the refrigerator, this oil solidified. The solid was removed by filtration and washed with cold acetone; yield 10.4 Gm. (72%) of light yellow solid, m. p. 177–180°. After three recrystallizations from 95% ethyl alcohol-acetone, a sample melted at 180–181°.

The starting amino ketone hydrochloride melted at 187°, and the melting point of a mixture of the above product and starting material was 165–176°.

Anal. Calcd. for $C_{12}H_{15}N_2O_3 \cdot HCl$: C, 54.45, H, 6.68. Found: C, 54.42, H, 6.75.

Generally, previous workers employing this general procedure have used a Vigreux column (12), a modified Widmer column (13), or Hahn condenser (14). However, in the present studies, ordinary ground glass equipment providing for ready interchange of an upright condenser with one turned downward for distillation served admirably.

3-Dialkylamino-1-phenyl-1-propanols (Table III).

—These compounds, synthesized by means of the foregoing procedure, are listed in Table III. Isolated as the hydrochlorides, they were recrystallized from alcohol-acetone mixtures. In each case, admixtures of the product with starting material caused a depression in melting point, indicating that the reduction was successful.

3-Dimethylamino-1-phenyl-1-propanol Hydrochloride (Table III, Compound 1).—This substance was synthesized through the foregoing method using aluminum isopropoxide and by Mannich and Heilner though catalytic hydrogenation, as indicated in Table I. The following sodium borohydride method has also been employed. It is based upon the general procedure of Chaikin and Brown (15).

A solution of 1.85 Gm. (0.05 mole) of sodium borohydride in 100 ml. of 50% methyl alcohol, kept at 20°, was placed in a 300-ml. round-bottomed flask, equipped with a stirrer, reflux condenser, and

dropping funnel. Meanwhile, 10.6 Gm. (0.05 mole) of β -dimethylaminopropiophenone hydrochloride (Table III, footnote a) was dissolved in a minimum volume of water, and the solution made basic with cold 20% sodium hydroxide. Ether extraction of the free base, removal of the ether, and addition of 75 ml. of methyl alcohol was followed by dropwise addition of the prepared solution to the ice-cooled sodium borohydride solution so as to maintain the temperature below 30°. After the addition was complete, the mixture was warmed to 45–50° in order to decompose the excess sodium borohydride. The solvent was removed *in vacuo* and the residue made basic with 6 N sodium hydroxide and extracted with ether. After drying over anhydrous sodium sulfate, hydrogen chloride gas was passed into the filtered ether solution to precipitate the amine hydrochloride. Yield 7.2 Gm. (67%), m. p. 128–132°. After recrystallization from acetone, the compound melted at 133–134°. There was no depression of melting point upon admixture with samples from other preparative methods.

α -Bromo- β -dialkylaminopropiophenones (Table IV).—The compounds of Table IV were prepared by the bromination of the appropriate ketonic Mannich base hydrobromides in glacial acetic acid, using the procedure of Land, Ziegler, and Sprague (10). In one case (compound 3) the hydrochloride was used. Compounds 1 and 3 were recrystallized from isopropyl alcohol; alcohol was used for all others.

REFERENCES

- (1) Burckhalter, J. H., and Johnson, S. H., *J. Am. Chem. Soc.*, **73**, 4835(1951).
- (2) Blicke, F. F., "Organic Reactions," Vol. I, John Wiley & Sons, Inc., New York, N. Y., 1942, ch. 10.
- (3) Long, L. M., and Troutman, H. D., *J. Am. Chem. Soc.*, **71**, 2469(1949).
- (4) Blicke, F. F., and Burckhalter, J. H., *ibid.*, **64**, 451(1942); Burckhalter, J. H., and Fuson, R. C., *ibid.*, **70**, 4184(1948).
- (5) (a) Burckhalter, J. H., and Johnson, S. H., *THIS JOURNAL*, **42**, 177(1953), Table I, footnote g. See also (b), Stamper, M., and Aycock, B. F., *J. Am. Chem. Soc.*, **76**, 2786(1954); (c) Wheatley, W. B., Fitzgibbon, W. E., and Cheney, *ibid.*, **76**, 4490(1954).
- (6) Ling Wei, Thesis, Master of Science, University of Kansas, 1950.
- (7) Wright, H. B., and Freifelder, M., *J. Am. Chem. Soc.*, **71**, 1513(1949).
- (8) Lutz, R. E., *et al.*, *J. Org. Chem.*, **12**, 617(1947).
- (9) For similar results see also footnote 5 in the article by Burckhalter, J. H., and Johnson, S. H., *J. Am. Chem. Soc.*, **73**, 4827(1951).
- (10) Land, A. H., Ziegler, C., and Sprague, J. M., *ibid.*, **69**, 125(1947).
- (11) Maxwell, C. E., *Org. Syntheses*, **23**, 30(1943).
- (12) Young, W. G., Hartung, W. H., and Crossley, F. S., *J. Am. Chem. Soc.*, **58**, 100(1936).
- (13) Baker, R. H., and Adkins, H., *ibid.*, **62**, 3305(1940).
- (14) Hahn, A., *Ber.*, **43**, 420(1910).
- (15) Chaikin, S. W., and Brown, W. G., *J. Am. Chem. Soc.*, **71**, 122(1949).

Antamebic Agents IV.* Quinolines and Acridines with Amino-Hydroxyalkylanilino Side Chains†

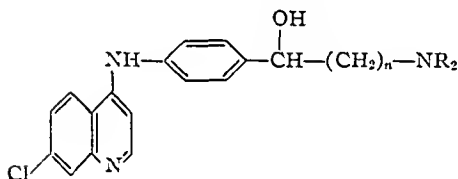
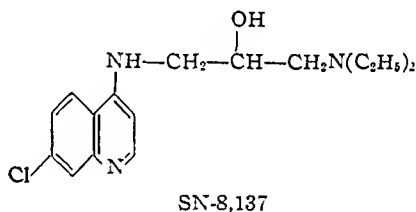
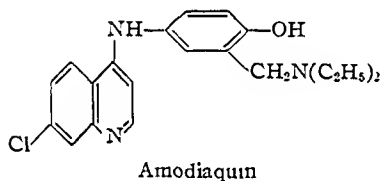
By W. LEWIS NOBLES,‡ VERLIN C. STEPHENS,§ LING WEI,||
and J. H. BURCKHALTER

Six quinolines and five acridines containing side chains incorporating some of the structural features of amodiaquin and SN-8,137 were synthesized as potential antiamebic or antimalarial agents. The antiamebic activity of the compounds tested was insufficient to offer promise. Two possessed antimalarial activity in chicks greater than quinine, but insufficient for further interest.

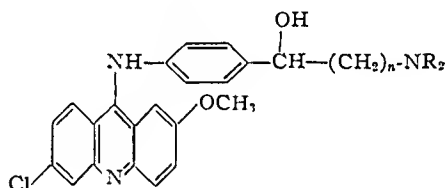
THE ACRIDINE ANTIMALARIAL, quinacrine, and the quinoline antimalarials, chloroquine and amodiaquin (Camoquin®), have shown activity against extra-intestinal amebiasis in hamsters and thus have been used for extra-intestinal amebiasis (1). As a part of a search for compounds with improved antiamebic activity, it was

malarial amodiaquin and the alcoholic hydroxyl as a favorable grouping in the antimalarial SN-8,137 (2), it appeared worth while to synthesize a number of agents (I-VI, inclusive) which would incorporate these special features of amodiaquin and SN-8,137.

Further, in view of the amebicidal effect of quinacrine, a few analogous acridines (VII, VIII, IX, and X) possessing the same side chains were also planned. Another one, indicated in the Experimental section as XI, differs from the other acridines in having a dimethylaminoalkanol chain attached *meta* to the benzene ring.



	n	NR ₂
I	1	Amino
II	1	Piperidyl
III	2	Dimethylamino
IV	2	Diethylamino
V	2	Pyrrolidyl
VI	2	Piperidyl

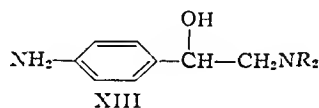
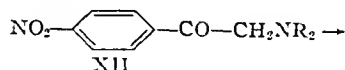
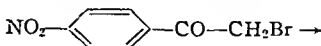


	n	NR ₂
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VIII	1	Piperidyl
IX	2	Diethylamino
X	2	Pyrrolidyl

decided to introduce certain chemical alterations into these antimalarial types

Owing to the presence of the benzene ring as an integral part of the side chain of the anti-

The synthetic scheme used for compounds I, II, VII, and VIII follows:



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† Received July 26, 1957, from the Laboratory of Pharmaceutical Chemistry, University of Kansas School of Pharmacy, Lawrence, Kan.

Portions of this paper were extracted from sections of the Ph. D. thesis of W. Lewis Nobles 1952 and the M. S. thesis of Ling Wei 1950.

‡ Fellow of The American Foundation for Pharmaceutical Education. Present address: School of Pharmacy, University of Mississippi, University, Miss.

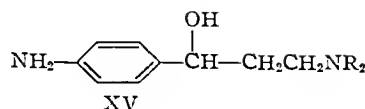
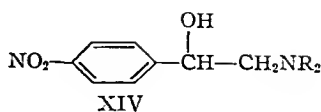
§ Parke, Davis and Co. Postdoctorate Fellow. Present address: Eli Lilly and Co. Indianapolis, Ind.

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Starting material *p*-nitrophenacyl bromide, was available, since it is required in one of the chemical processes for chloramphenicol. The general procedure, adapted from the literature (3), consisted of mixing *p*-nitrophenacyl bromide with two equivalents of secondary amine in benzene or ether solution (4), allowing the mixture to stand for several hours, removing the precipitated by-product amine hydrobromide, drying the solution of the product over an inorganic drying agent, and finally precipitating the product (XII) as the hydrochloride. In practice, the by-product amine hydrobromide was obtained in nearly quantitative yield, but only a brown tarry product resulted. It was eventually found that the desired *p*-nitro- α -(1-piperidyl)-acetophenone could be prepared if the reaction were allowed to proceed for only a short while, and the hydrochloride were formed as quickly as possible to prevent degradation of the desired product. Also, a nitrogen atmosphere appeared to be favorable. Under such circumstances, it is not surprising that the analytical data of the piperidyl and diethyl intermediates (XIIa and b) were not entirely satisfactory. In the case of XIIb, decomposition during recrystallization occurred even though it was in the form of the hydrochloride; therefore, it was not employed for further synthesis.

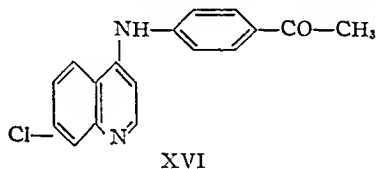
p-Nitro- α -aminoacetophenone, the third intermediate of the type, has already been prepared by Long and Troutman (3). Its reduction and that of *p*-nitro- α -(1-piperidylamino)-acetophenone took place readily in very dilute hydrochloric acid to the diamines (XIII) by using platinum oxide catalyst and about three atmospheres of hydrogen. In the case of the piperidyl compound, it was necessary to keep the aqueous solution warm by means of a heat lamp to avoid the precipitation of an intermediate product, which prevented further reduction. Isolation of the intermediate diamine hydrochlorides was not feasible because of apparent decomposition in the air, but isolation was actually unnecessary since, after removal of the catalyst by filtration, the solution possessed the proper pH for reactivity with either 4,7-dichloroquinoline or 6,9-dichloro-2-methoxyacridine. This procedure has been successfully employed before for the synthesis of amodiaquin type compounds (5).

For the synthesis of the quinolines (III–VI inclusive) and acridines (IX and XI), 3-dialkylamino-1-(nitrophenyl)-1-propanols (XIV), made available from previous studies (6), were reduced catalytically to the diamino alcohols (XV), which were allowed to react directly, as in the case of



XIII, with either 4,7-dichloroquinoline or 6,9-dichloro-2-methoxyacridine.

Earlier, unsuccessful attempts to obtain quinolines of the type III to VI were made through preparation of 4-(7-chloro-4-quinolylamino)-acetophenone (XVI), as well as the isomeric 3-substituted acetophenone (XVII). Excellent yields



of XVI or XVII were obtained from a condensation of 4,7-dichloroquinoline and *p*- or *m*-aminoacetophenone in an acidic medium. However, these substituted ketones could not be made to undergo the Mannich reaction to give the amino ketones required for the synthesis of Type III–VI compounds.

Pharmacological Results.¹—Quinolines I, II, and VI were active *in vitro* against *E. histolytica*, but only at 1:500 dilution. Tested in rats as a possible intestinal amebicide, I was found to be slightly active but not promising. Against hepatic amebiasis in hamsters, I was less active than chloroquine. Acridines VII, VIII, IX, X, and XI showed *in vitro* activity at 1:500 dilution, and *in vivo* in rats, the same compounds were described "as active but not promising."

Quinoline I and acridines VII, IX, X, and XI had quinine equivalents (quinine = 1) of, respectively, 2.4, 0.45, 1.5, 0.8, and 0.3 against *Plasmodium lophurae* in chicks, but these activities were not considered to be of interest when compared with currently accepted antimalarial drugs.

EXPERIMENTAL²

p-Nitro- α -(1-piperidyl)-acetophenone Hydrochloride Monohydrate (XIIa).—A solution of 34 Gm. (0.4 mole) of piperidine in 75 ml. of absolute ether was added dropwise, with vigorous stirring and under a slow stream of nitrogen, over a period

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² Melting points are uncorrected.

Antamebic Agents IV.* Quinolines and Acridines with Amino-Hydroxyalkylanilino Side Chains†

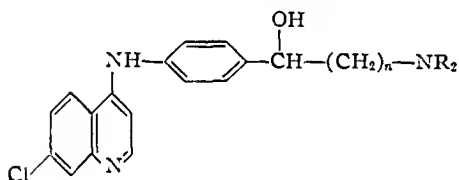
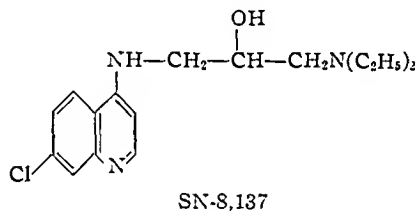
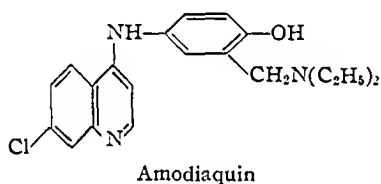
By W. LEWIS NOBLES,‡ VERLIN C. STEPHENS,§ LING WEI,||
and J. H. BURCKHALTER

Six quinolines and five acridines containing side chains incorporating some of the structural features of amodiaquin and SN-8,137 were synthesized as potential antiamebic or antimalarial agents. The antiamebic activity of the compounds tested was insufficient to offer promise. Two possessed antimalarial activity in chicks greater than quinine, but insufficient for further interest.

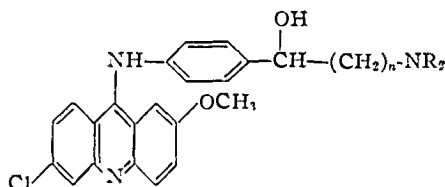
THE ACRIDINE ANTIMALARIAL, quinacrine, and the quinoline antimalarials, chloroquine and amodiaquin (Camoquin®), have shown activity against extra-intestinal amebiasis in hamsters and thus have been used for extra-intestinal amebiasis (1). As a part of a search for compounds with improved antiamebic activity, it was

malarial amodiaquin and the alcoholic hydroxyl as a favorable grouping in the antimalarial SN-8,137 (2), it appeared worth while to synthesize a number of agents (I-VI, inclusive) which would incorporate these special features of amodiaquin and SN-8,137.

Further, in view of the amebicidal effect of quinacrine, a few analogous acridines (VII, VIII, IX, and X) possessing the same side chains were also planned. Another one, indicated in the Experimental section as XI, differs from the other acridines in having a dimethylaminoalkanol chain attached *meta* to the benzene ring.



	n	NR ₂
I	1	Amino
II	1	Piperidyl
III	2	Dimethylamino
IV	2	Diethylamino
V	2	Pyrrolidyl
VI	2	Piperidyl

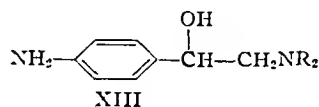
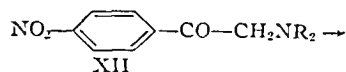
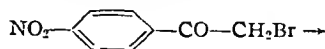


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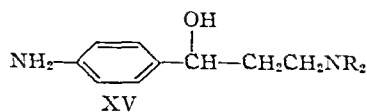
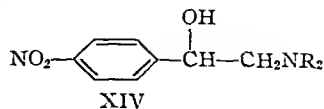
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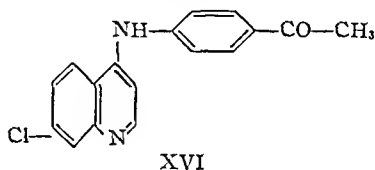
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of a half hour to a suspension of 48.8 Gm. (0.2 mole) of *p*-nitrophenacyl bromide (3) in 400 ml of absolute ether. Stirring was continued for about five minutes after the addition was complete, and by-product piperidine hydrochloride in nearly quantitative amount was quickly removed by filtration and washed with ether. Dry hydrogen chloride in excess was immediately bubbled into the filtrate. The precipitated product was collected on a funnel and washed well with acetone. It was air dried to give 51 Gm (84% yield) of crude material which melted at 160–166° decompn. By recrystallization from 200 ml of methanol, 39 Gm (65% yield) of product was obtained, m p. 177–181° decompn. Further recrystallization from methanol-acetone did not alter the melting point.

An attempt to analyze this compound for ionic chloride using silver nitrate resulted in the formation of free silver.

Anal. Calcd for $C_{13}H_{16}N_2O_3 \cdot HCl \cdot H_2O$: C, 51.57; H, 6.32; Found: C, 52.25; H, 6.60.

***p*-Nitro- α -diethylaminoacetophenone Hydrochloride Monohydrate (XII b).**—By the foregoing procedure, 12.2 Gm (0.05 mole) of *p*-nitrophenacyl bromide and 7.3 Gm (0.1 mole) of diethylamine gave 12 Gm of crude product, m p about 150° decompn. It was recrystallized with difficulty from a methanol-acetone mixture and from isopropyl alcohol. After four recrystallizations, it melted at 165–167° decompn. Decomposition appeared to occur readily in hot solvents.

Anal. Calcd for $C_{15}H_{18}N_2O_3 \cdot HCl \cdot H_2O$: C, 49.57; H, 6.59; Found: C, 50.01; H, 6.00.

Reduction to Diamines (Types XIII and XV).—A suspension of 12 Gm (0.056 mole) of *p*-nitro- α -aminoacetophenone hydrochloride (3) in a solution of 6 ml of hydrochloric acid and 200 ml of water was warmed and then subjected to platinum oxide reduction in a Parr apparatus. Within two hours the theoretical amount of hydrogen was usually absorbed. The catalyst was then removed by filtration, and the filtrate used directly in subsequent reactions.

Reduction of the piperidyl analog (XIIa) was carried out in the same fashion except that it was necessary to keep the suspension hot during the reduction by means of a heat lamp, in order to prevent precipitation of an intermediate product.

Type XIV intermediates in the form of the hydrochlorides (6) were suspended in alcohol and reduced catalytically using Adams' catalyst to type XV diamines.

Compounds from Type XII Intermediates

2-Amino-1-[*p*-(7-Chloro-4-quinolylamino)-phenyl]-1-ethanol Dihydrochloride (I).—The filtrate from the reduction of *p*-nitro- α -aminoacetophenone, containing diamine (XIII) in acid solution, and 10 Gm (0.05 mole) of 4,7-dichloroquinoline was heated on steam bath for thirty minutes, according to the general procedure of Burckhalter, *et al* (5). After filtration, the solution was concentrated to a thick brown sirup. It was then warmed briefly with absolute alcohol and the solution allowed to cool overnight to give a yellow product, 14.5 Gm (73% yield), m p >300°. It was recrystallized from methanol.

Anal. Calcd for $C_{17}H_{18}Cl_2N_3O \cdot 2HCl \cdot \frac{1}{2}H_2O$: C, 51.60; H, 4.84; Found: C, 52.02; H, 5.06.

2-Amino-1-[*p*-(6-chloro-2-methoxy-9-acrydylamino)-phenyl]-1-ethanol Dihydrochloride (VII).—The filtrate from the reduction of 12 Gm of *p*-nitro- α -aminoacetophenone hydrochloride was heated with an alcohol suspension of 6,9-dichloro-2-methoxyacridine, following the foregoing general procedure. However, concentration of the filtrate was not necessary in giving 21 Gm. (80% yield) of bright orange-colored product, m p >300°. Recrystallization from very dilute hydrochloric acid³ yielded 18 Gm (70%) of product.

Anal. Calcd for $C_{27}H_{20}ClN_3O_2 \cdot 2HCl \cdot 2H_2O$: C, 50.73; H, 5.42; Found: C, 50.35; H, 5.66.

2-1'-Piperidyl-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-ethanol (II).—The filtrate from the reduction of 15.1 Gm (0.05 mole) of XII b was treated with 0.05 mole of 4,7-dichloroquinoline, as in the production of I. When no product precipitated from the reaction mixture, it was rendered basic with ammonia solution. The resulting precipitate was collected, washed with water and then acetone to remove any unreacted dichloroquinoline. Two recrystallizations of an impure product gave 8 Gm (42% yield) of white base (II), m p 191–192°.

Anal. Calcd for $C_{22}H_{21}ClN_3O \cdot C$, 69.19; H, 6.33; Found: C, 68.72; H, 6.74.

2-1'-Piperidyl-1-[*p*-(6-chloro-2-methoxy-9-acrydylamino)-phenyl]-1-ethanol Dihydrochloride (VIII).—The filtrate from the reduction of 14.2 Gm. (0.047 mole) of XII b was treated with 0.05 mole of the chloroacridine, as in VII, to give 22.5 Gm (90% yield) of product, m p. 195–200° decompn. Recrystallization from 2% hydrochloric acid³ gave 18 Gm (72% yield) of bright red II, m p. 207–210°. After two further recrystallizations, m p. was 210–213°. For analysis, a small sample was dried for three days over phosphorus pentoxide.

Anal. Calcd for $C_{27}H_{23}ClN_3O_2 \cdot 2HCl$: Cl, 13.26; Found: Cl, 13.24.

Compounds from Type XIV Intermediates

3-Dimethylamino-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-propanol (III).—Three grams (0.011 mole) of 3-dimethylamino-1-(*p*-nitrophenyl)-propanol hydrochloride (6) was suspended in absolute alcohol and reduced catalytically using Adams' catalyst. The clear solution thus obtained was treated with a slight excess of alcoholic hydrogen chloride and then filtered to remove the catalyst. An equivalent amount, 2.2 Gm (0.011 mole), of 4,7-dichloroquinoline was added to the filtrate and the resulting mixture was heated at reflux temperature for about two hours, during which time a clear solution resulted. The reaction mixture was made basic with 28% ammonium hydroxide and 4 Gm (78% yield) of a light yellow solid precipitated, m p 207–212° decompn. It was recrystallized from dilute alcohol, m p 218–220° decompn.

Anal. Calcd for $C_{20}H_{22}ClN_3O$: C, 67.49; H, 6.23; Found: C, 68.07; H, 6.77.

3-Diethylamino-1-[*p*-(7-chloro-4-quinolylamino)-phenyl]-1-propanol (IV).—Using the foregoing procedure, the appropriate reactants (6) gave 58% of light yellow solid (IV), m p 199–201° decompn (203–204 decompn. after recrystallization from dilute alcohol).

³ Recrystallization from water is possible but the presence of acid facilitates filtration and drying.

Anal.: Calcd. for $C_{22}H_{26}ClN_3O$: C, 68.82; H, 6.83. Found: C, 68.20; H, 7.19.

3-Diethylamino-1-[p-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-propanol Dihydrochloride Monohydrate (IX).—Using the procedure of III, except that the hydrochloride was isolated upon cooling the solution after reduction, the appropriate reactants (6) gave 69% yield of orange-colored IX, m. p. 192–195° decompn. (195–197° decompn. after recrystallization from alcohol-acetone).

Anal.: Calcd. for $C_{27}H_{30}ClN_3O_2 \cdot 2HCl \cdot H_2O$: C, 58.43; H, 6.18. Found: C, 58.30; H, 6.48.

3-1'-Pyrrolidyl-1-[p-(7-chloro-4-quinolylamino)-phenyl]-1-propanol Dihydrochloride Hydrate (V).—Using the procedure of III, the appropriate reactants (6) gave 82% yield of crude free base of V, m. p. 188–191° decompn. When recrystallization from dilute alcohol failed to yield an analytically pure sample, the compound was dissolved in dry ether. An ether-insoluble portion was removed by filtration, and the hydrochloride was precipitated by passage of dry hydrogen chloride into the solution. By dissolving in alcohol and reprecipitating through the addition of acetone, the flocculent precipitate was crystallized. Two recrystallizations from alcohol-acetone gave white product V, m. p. 141–146° decompn.

Anal.: Calcd. for $C_{22}H_{24}ClN_3O \cdot 2HCl \cdot \frac{1}{2}H_2O$: C, 52.85; H, 6.26. Found: C, 52.52; H, 6.68.

3-1'-Pyrrolidyl-1-[p-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-propanol Dihydrochloride (X).—Using the procedure of III except that the hydrochloride was isolated upon cooling the solution after reduction, the appropriate reactants (6) gave 76% yield of orange-colored X, m. p. 190–193° decompn. Recrystallized from alcohol-acetone, it melted at 194–195° decompn.

Anal.: Calcd. for $C_{27}H_{28}ClN_3O_2 \cdot 2HCl \cdot \frac{1}{2}H_2O$: C, 57.70; H, 6.01. Found: C, 57.60; H, 6.38.

3-1'-Piperidyl-1-[p-(7-chloro-4-quinolylamino)-phenyl]-1-propanol Dihydrochloride (VI).—Using the procedure of III and the appropriate intermediates (6), the product was obtained in the form of an amorphous mass which could not readily be crystallized. The material was dissolved in ether, the ether solution dried over potassium carbonate, and the hydrochloride made by passing dry hydrogen chloride into the filtered ether solution. The yellow crystallizing precipitate VI was obtained in 46% yield, m. p. 278–281° decompn. Recrystallized from alcohol-acetone, it melted at 283–284° decompn.

Anal.: Calcd. for $C_{23}H_{26}ClN_3O \cdot 2HCl$: C, 58.92; H, 6.02. Found: C, 58.67; H, 6.12.

3-Dimethylamino-1-[m-(6-chloro-2-methoxy-9-acridylamino)-phenyl]-1-propanol Dihydrochloride (XI).—Using the procedure of III except that the

hydrochloride was isolated upon cooling the solution after reduction, the appropriate reactants (6) gave 67% yield of orange-colored XI, m. p. 174–178° decompn. It was recrystallized from alcohol-acetone, m. p. 179–180° decompn.

Anal.: Calcd. for $C_{25}H_{26}ClN_3O_2 \cdot 2HCl \cdot \frac{1}{2}H_2O$: C, 54.30; H, 6.07. Found: C, 54.20; H, 6.00.

4-(7-Chloro-4-quinolylamino)-acetophenone (XVI).—A mixture of 13.5 Gm. (0.1 mole) of *p*-aminoacetophenone, 19.8 Gm. (0.1 mole) of 4,7-dichloroquinoline, 100 ml. of alcohol, and 0.5 ml. of concentrated hydrochloric acid was heated at reflux temperature for two hours. After cooling the reaction mixture, the bright yellow product (XVI) hydrochloride was isolated in nearly quantitative yield, m. p. 272–273° decompn. Recrystallization from alcohol changed the melting point to 281–282° decompn.

Anal.: Calcd. for $C_{17}H_{13}ClN_2O \cdot HCl$: C, 61.23; H, 4.20. Found: C, 61.57; H, 4.12.

A quantity of the hydrochloride was treated while stirring with excess alcoholic ammonium hydroxide solution to give the free base XVI. The product was collected and washed thoroughly with distilled water until chloride ion was absent from the filtrate. Recrystallized from methyl alcohol, the yellowish XVI was obtained in 88% yield, m. p. 222°.

Anal.: Calcd. for $C_{17}H_{13}ClNO \cdot \frac{1}{2}H_2O$: C, 66.81; H, 4.62. Found: C, 66.50; H, 4.42.

3-(7-Chloro-4-quinolylamino)-acetophenone (XVII).—Employing *m*-aminoacetophenone in place of its para-isomer in the foregoing procedure, a 90% yield of yellow (XVII) hydrochloride was obtained, m. p. 301° decompn.

Anal.: Calcd. for $C_{17}H_{13}ClN_2O \cdot HCl$: Cl (ionic), 10.65. Found: Cl, 10.52.

The hydrochloride was converted to the yellowish free base XVII, as with XVI in 93% yield, m. p. 190°.

Anal.: Calcd. for $C_{17}H_{13}ClN_2O$: C, 68.79; H, 4.42. Found: C, 68.41; H, 4.40.

REFERENCES

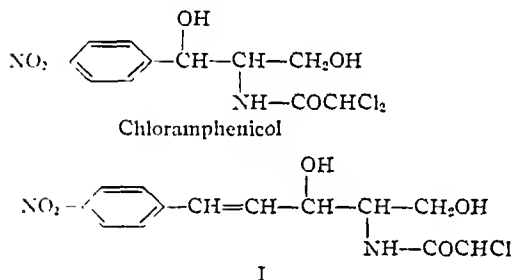
- (1) Thompson, P. E., and Reinertson, J. W., *Am. J. Trop. Med.*, **31**, 715(1951); Anderson, H. H., and Hansen, E. L., *Pharmacological Revs.*, **2**, 402(1950); "New and Non-official Remedies," 1957, p. 153.
- (2) "A Survey of Antimalarial Drugs, 1941–1945," F. Y. Wiselogle, Editor, J. W. Edwards, Ann Arbor, Mich., 1946, Vol. I, p. 392.
- (3) Long, L. M., and Troutman, H., *J. Am. Chem. Soc.*, **71**, 2473(1949).
- (4) Marvel, C. S., and duVigneaud, V., *ibid.*, **46**, 2093(1924); Lutz, R. E., *et al.*, *J. Org. Chem.*, **12**, 656(1947).
- (5) Burckhalter, J. H., *et al.*, *J. Am. Chem. Soc.*, **70**, 1363(1948).
- (6) Nobles, W. L., and Burckhalter, J. H., *THIS JOURNAL*, **47**, 77(1958).

Attempted Synthesis of a Vinylog of Chloramphenicol*

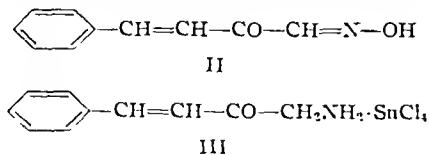
By W. LEWIS NOBLES† and J. H. BURCKHALTER

Applying essentially the synthetic procedures of Long and Troutman, which led to chloramphenicol, we have attempted to synthesize a vinylog of the antibiotic in order to study the application of the theory of vinylogy to pharmacological results. However, failure to achieve the desired synthesis has suggested that even chemically the theory does not hold entirely, especially in regard to an apparently high specificity of structure which allows monohydroxymethylation in certain chloramphenicol intermediates and not in their vinylogs.

IT IS WELL KNOWN that the chemical reactivity of a compound and its vinylog may be similar (1). In further study of the theory that a vinylog may possess pharmacological action similar to the parent drug (2), we proposed to attempt the synthesis of a vinylog (I) of chloramphenicol, a structure differing from the antibiotic only by a vinyl grouping. Although the synthesis of I was not achieved, we wish to report the results which were obtained.



The synthetic scheme was patterned after the approach of Long and Troutman to chloramphenicol (3). The early styryl intermediates had been made over fifty years ago by Sir Robert Robinson for an entirely different purpose (4). The latter prepared oximinomethyl styryl ketone (II) from benzalacetone and isoamyl nitrile, and then reduced it with stannous chloride and hydro-

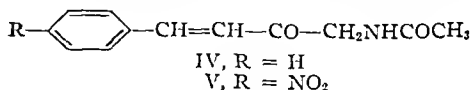


chloric acid to the stannichloride complex (III). However, it was found that the previously de-

veloped procedures, while satisfactory for small quantities of reactants, were unsuited for larger amounts. For example, in the preparation of II, a temperature as low as 20° was necessary, yet near that temperature benzalacetone crystallized from solution. But, if the solution of benzalacetone in Skelly B was prepared by melting the ketone and adding it to the solvent on the night prior to the morning during which the reaction was carried out, the solution would cool slowly to room temperature (16–20°) without depositing crystals of benzalacetone. Further, in contrast with the original work involving smaller quantities (4), in order to prevent decomposition of II, it was important to keep the mixture cooled with stirring for two hours after all the reagent had been added, instead of allowing it to stand at room temperature.

The stannous chloride reduction of oxime II to III, employing the original procedure (4), afforded difficulty. In our hands, direct addition of II to stannous chloride in acid caused the evolution of considerable heat and resulted in the formation of large, dark lumps of solid which were difficult to purify. Upon the suggestion of Dr. S. H. Johnson, of This Laboratory, these difficulties were obviated by adding the oxime as an alcoholic solution.

Acetylation of the tin complex salt (III) in acetic acid using sodium acetate as a buffer, as prescribed for its benzoylation (4), and employing acetic anhydride or acetyl chloride or mixtures of the two, gave only small amounts of IV. However, the procedure of Long and Troutman (3) using sodium hydroxide and acetic anhydride



resulted in consistently good yields of 1-acetamido-4-phenyl-3-buten-2-one (IV). This compound was then nitrated to give a 50% yield of V.

* Received July 26, 1957, from the Laboratory of Pharmaceutical Chemistry, University of Kansas School of Pharmacy, Lawrence, Kan.

† Extracted from a portion of the Ph. D. thesis of W. Lewis Nobles, 1952.

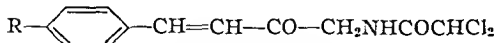
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Unlike the nitration of benzalacetone (2), only the *para*-isomer was isolated.

Attempts to hydroxymethylate V resulted only in the recovery of starting material. This might be attributed to the lack of solubility of V in solvents customarily used in the reaction. While V is soluble in boiling alcohol, Long and Troutman (3) have indicated that the temperature should be kept between 35 and 40° for similar hydroxymethylations.

In view of the failure to hydroxymethylate V, attempts were made to introduce the grouping into IV which lacks the nitro group and which is more soluble in the required solvents. It was thought that the diacetyl derivative could then be *para*-nitrated in a step toward obtaining I. However, no less than thirty unsuccessful attempts were made to monohydroxymethylate IV. The use of sodium or potassium carbonate as catalyst at room temperature led to no reaction. When the temperature was raised to 50–60°, the bis-methane structure was apparently formed. The product of the reaction using sodium bicarbonate at 35–40° was apparently the substance containing two hydroxymethyl groups. The use of one, two, or four equivalents of formaldehyde still gave the same compound. Other workers, notably Pain and Slack (5) and Hays and associates (6), have commented upon the difficulty of attaining such monomethylol derivatives. The latter workers concluded that the reaction will not as a general rule yield them. Our results are in accord with this conclusion.

Still another approach, patterned after the studies of Long and Troutman (7), was followed. IV and V were hydrolyzed in dilute hydrochloric acid to the corresponding amine hydrochlorides. The yields were neither good nor consistent, and considerable decomposition occurred. Nevertheless, these salts, designated as VI and VII in the Experimental section, were treated with dichloroacetyl chloride in the presence of triethylamine to yield VIII and IX. Though a satis-



VIII, R = H
IX, R = NO₂

factory elementary analysis of IX was not obtained, infrared studies of the compound conducted by Mr. Bruce Scott and Dr. George Moersch, of Parke, Davis & Co., Research Division, indicated the structure to be correct. The analytical data suggest that the starting material dichloroacetyl chloride was contaminated with monochloroacetyl chloride.

Finally, an attempt was made to monohydroxy-

methylate VIII. Here, unlike the previous experiments, the desired derivative (X) was obtained. But, in view of the poor yields obtained in the sequence of reactions, it was decided to discontinue the studies at this point.

Since the desired vinylog of chloramphenicol was not obtained, conclusions based upon pharmacological activity are impossible to draw. However, in regard to chemical adherence to the theory of vinylogy, it is felt that there was a lack of complete conformity because of our failure to monohydroxymethylate vinylogs IV and V, whereas the same conditions have been shown to give positive results with the parent compounds (3).

EXPERIMENTAL¹

Oximinomethyl Styryl Ketone (II).—Into a three-liter, three-necked flask equipped with a mechanical stirrer was placed 1.5 liters of Skelly Solve B. One hundred grams (0.69 mole) of benzalacetone was melted on the steam bath and dissolved therein. The flask was allowed to come slowly to room temperature, surrounded by a water bath. To this solution was then added 225 ml. of isoamyl nitrite (freshly prepared) and 40 ml. of concentrated hydrochloric acid in alternate portions. The temperature was maintained near 20° by the addition of small amounts of ice to the surrounding water bath. After the addition was complete, the flask was surrounded by an ice-salt bath and stirring continued for another two hours. The solid thus obtained was removed by filtration and washed alternately with benzene and water until the washings were colorless. The weight of air-dried material was 82 Gm. (65% yield). The product was recrystallized from methyl alcohol-water to yield a solid that melted at 143°. Robinson and Foulds (4) reported no melting point for this compound. In regard to yield, they indicated only that it was "excellent." Recrystallization was not routinely employed, since experience indicated that the air-dried material was pure enough for the subsequent reaction.

Aminomethyl Styryl Ketone Stannichloride (III).—Oximino styryl methyl ketone (40 Gm., 0.23 mole) was dissolved in 95% ethyl alcohol. A solution of 110 Gm. of stannous chloride in 300 ml. of concentrated hydrochloric acid was prepared, cooled and placed in a one-liter, three-necked flask equipped with a mechanical stirrer. The flask was surrounded by an ice bath and the alcoholic solution of the oxime added slowly with stirring. Upon the completion of the addition, the ice bath was removed and the stirring continued for six hours. The solid was removed by filtration and washed thoroughly with alcoholic hydrogen chloride. A light tan solid was obtained upon air drying this material. Eighty-two grams (80% yield) of the tin complex was thus obtained.

1-Acetamido-4-phenyl-3-buten-2-one (IV).—To a three-liter flask equipped with an efficient stirrer, a thermometer, and a dropping funnel, was added the

¹ Melting points are uncorrected.

tin complex salt obtained from 35 Gm (0.2 mole) of oximinomethyl styryl ketone and 250 ml of hot water. The mixture was stirred until a clear solution was produced. The flask was then cooled to -5° . Twenty-five grams of acetic anhydride was added in one portion, and the resulting mixture stirred rapidly while a cold solution of 42 Gm of sodium hydroxide in 100 ml of water was added at such a rate as to keep the temperature below 10° . When about 75 ml of the sodium hydroxide solution had been added and the mixture was almost alkaline, 7 Gm of acetic anhydride was poured in. Addition of the hydroxide was completed and stirring maintained for thirty minutes at 0° .

The mixture was saturated with sodium chloride and extracted five times with 500 ml portions of ethyl acetate. The combined extracts were washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated to 200 ml by removal of solvent. To the hot concentrate was added 180 ml of Skelly B. The mixture was cooled to 5° and filtered. The yield was 34 Gm (81%), m p $118-123^{\circ}$. An analytical sample was obtained by recrystallization from water. It melted at 127° .

Anal. Calcd for $C_{17}H_{13}NO_2$: C, 71.90, H, 6.45. Found: C, 71.74, H, 6.36.

1-Acetamido-4-(*p*-nitrophenyl)-3-buten-2-one (V).—To a 200 ml, three-necked flask, equipped with a mechanical stirrer and thermometer, was added 60 ml of sulfuric acid. The flask was surrounded by an acetone dry ice bath and the temperature lowered to -20° by the addition of pieces of dry ice. To this was then slowly added 20.3 Gm (0.1 mole) of IV, care being taken to insure that the temperature did not rise above -10° during the addition. When all the solid had been added and the temperature again lowered to -20° , a nitrating solution of 6.4 ml of concentrated nitric acid (sp gr 1.42) and 10 ml of concentrated sulfuric acid was

added dropwise. The mixture in the flask became rather dark and very viscous. The mixture was stirred for fifteen minutes after the addition of the last portion of acid, the cooling bath having been removed during this period of stirring. The mixture was then poured into one liter of cracked ice and water, and stirred for two hours. A yellow, rather pasty solid formed at first, but this soon became more granular in appearance. The solid was removed by filtration and washed with water to remove the traces of acid present. It was then allowed to air dry overnight, yield 12.2 Gm (50%), m p $146-155^{\circ}$. A sample for analysis was purified by repeated recrystallization from ethyl acetate-Skelly B. The purified product is fluffy in appearance and light yellow in color, m p 171° .

Anal. Calcd for $C_{17}H_{13}N_2O_4$: C, 58.05, H, 4.87. Found: C, 58.32, H, 5.01.

For Compound A $C_6H_5-CH=CH-CO-C(NHCOCH_3)(CH_2OH)_2$

Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.86, H, 6.51. Found: C, 63.87, H, 6.61.

For Compound B $[C_6H_5-CH=CH-CO-CH(NHCOCH_3)]_2-CH_2$

Anal. Calcd for $C_{25}H_{26}N_2O_4 \cdot \frac{1}{2}H_2O$: C, 70.23, H, 6.40. Found: C, 70.25, H, 6.66.

1-Amino-4-phenyl-3-buten-2-one Hydrochloride (VI).—Eight and one tenth grams (0.04 mole) of the corresponding acetamido derivative was heated for two hours on the steam bath with 100 ml of 5% hydrochloric acid. The solid dissolved shortly after the heating began. The solution gradually became dark red in color and a dark oil formed on the bottom of the flask. The mixture was cooled slowly to room temperature and then the hydrolysate was twice extracted with ether to remove any neutral material. The aqueous solution of the hydrochloride was then taken to dryness *in vacuo*. The solid thus obtained was filtered and washed with acetone-ether, yield 3 Gm (39%). The crude

Catalyst	Solvent	Time, hr	Equivalents of Formaldehyde	Product and Yield
NaHCO ₃	CH ₃ OH—HOH	2	1	A (40%)
NaHCO ₃	HOH	2	2	A (75-85%)
NaHCO ₃	HOH	2	4	A (75-85%)
Na ₂ CO ₃	CH ₃ OH	2	2	B (poor)
NaHCO ₃	CH ₃ OH	12	2 (para-formal)	B (poor)
K ₂ CO ₃	C ₂ H ₅ OH	4	2	Undetermined

added dropwise. The mixture in the flask became rather dark and very viscous. The mixture was stirred for fifteen minutes after the addition of the last portion of acid, the cooling bath having been removed during this period of stirring. The mixture was then poured into one liter of cracked ice and water, and stirred for two hours. A yellow, rather pasty solid formed at first, but this soon became more granular in appearance. The solid was removed by filtration and washed with water to remove the traces of acid present. It was then allowed to air dry overnight, yield 12.2 Gm (50%), m p $146-155^{\circ}$. A sample for analysis was purified by repeated recrystallization from ethyl acetate-Skelly B. The purified product is fluffy in appearance and light yellow in color, m p 171° .

solid was dissolved in an acetone-isopropyl alcohol solution, treated with charcoal, filtered, and set aside in the refrigerator to cool. White crystals, m p $184-189^{\circ}$, were obtained. On further recrystallization from acetone-isopropyl alcohol, the melting point was raised to 193° .

Anal. Calcd for $C_{10}H_{11}NO \cdot HCl$: Cl, 17.94. Found: Cl, 18.12.

1-Amino-4-(*p*-nitrophenyl)-3-buten-2-one Hydrochloride (VII).—Seven and five tenths grams (0.03 mole) of 1-acetamido-4-(*p*-nitrophenyl)-3-buten-2-one (V) was heated with 125 ml of 5% hydrochloric acid on the steam bath for two and one-half hours. Slowly a red solution formed, but simultaneously a considerable amount of brown tar was deposited in the bottom of the flask. The mixture was filtered while warm and then set aside to cool. A yellow solid, 3 Gm (41% yield), m p $262-266^{\circ}$ (decolorn),

was thus obtained. This solid was then recrystallized from dilute ethyl alcohol to which a few drops of concentrated hydrochloric acid had been added. It melted at 276° (decomp.). The compound begins to darken and shrink in the region of 210–215°, but true melting does not occur until the higher temperature is reached.

Anal. Calcd. for $C_{10}H_{10}N_2O_4 \cdot HCl$: Cl, 14.61. Found: Cl, 14.74.

1-Dichloroacetamido-4-phenyl-3-buten-2-one (VIII).—Five and nine-tenths grams (0.03 mole) of VI and 5.1 Gm. (0.035 mole) of dichloroacetyl chloride were mixed, together with 100 ml. of dry benzene, in a 300-ml. flask equipped with a reflux condenser, mechanical stirrer, and dropping funnel. The mixture was cooled and stirred while 6.67 Gm. (0.67 mole) of triethylamine was added over a period of fifteen minutes. Toward the end of the addition of the amine, the mixture thickened and the cooling bath was removed. Stirring was continued for another hour. The mixture was then heated to reflux and filtered while hot. As the filtrate cooled a quantity of solid product separated and this was removed by filtration. The filtrate was then evaporated to dryness by passing a current of air over it. The residue thus obtained was washed first with Skelly B and then with water. This residue was then combined with the solid previously obtained by filtration and both were recrystallized from alcohol-water; yield 3.8 Gm. (47%), m. p. 128–131°. An analytical sample was obtained by two additional recrystallizations from benzene; m. p. 133–134°.

Anal. Calcd. for $C_{12}H_{11}Cl_2NO_2$: C, 52.96; H, 4.07. Found: C, 53.23; H, 4.13.

1-Dichloroacetamido-4-(*p*-nitrophenyl)-3-buten-2-one (IX).—Seven and one-half grams (0.03 mole) of VII and 5.1 Gm. (0.035 mole) of dichloroacetyl chloride were mixed together with 160 ml. of dry benzene in a 300-ml. flask equipped with a mechanical stirrer, dropping funnel, and reflux condenser. The mixture was surrounded by an ice bath and cooled and stirred while 6.7 Gm. (0.065 mole) of triethylamine was added over a period of twenty minutes. Stirring was continued for a total of two hours; the cooling bath having been removed toward the end of the addition of the triethylamine. The mixture was then heated to reflux and filtered hot. The filtrate was concentrated *in vacuo*. The residue was washed, first with Skelly B and then with water. It was dissolved in 95% ethyl alcohol by warming, treated with charcoal, and filtered while hot. The filtrate was made slightly turbid by the addition of a few drops of water. This was again filtered and set aside to cool. The yellow solid which formed was removed by filtration; yield 3 Gm. (30%), m. p. 178–182°. An analytical sample was prepared by repeated recrystallization from benzene.

Three different samples were prepared by the

above method and each was analyzed. A typical result is given.

Anal. Calcd. for $C_{12}H_{10}Cl_2N_2O_4$: C, 45.44; H, 3.17; Cl, 22.36. Found: C, 46.35; H, 3.65; Cl, 20.15.

TABLE I.—INFRARED SPECTRAL DATA ON COMPOUND IX

Wavelength, μ , Absorption Peaks (IX)	Wavelength ranges, μ , of Compounds Contg. Similar Functional Groups ^b
N—H.....3.01	2.88–3.00
C=O, keto.....5.91	5.81–5.99
C=O, amide I ^a ...6.00	5.99–6.25
C=C.....6.18	6.02–6.11
C ₆ H ₅ C=C.....6.28	6.30–6.35
Amide II ^a6.45	6.39–6.67
Nitro I ^a6.64	6.23–6.56
Nitro II ^a7.45	7.20–7.60

^a One of two characteristic bands in this region.

^b As listed by H. M. Randall, *et al.*, in "Infrared Determination of Organic Substances," D. Van Nostrand Co., Inc., New York, 1949, p. 20.

In addition to this information, Dr. Moersch of Parke, Davis and Co. stated that the ultraviolet examination of IX gives an absorption very similar to *p*-nitrocinnamaldehyde, which would be expected for a structure of this type.

2-Dichloroacetamido-1-hydroxy-5-phenyl-4-penten-3-one (X).—A mixture of 1.35 Gm. (0.005 mole) of VIII, 10 ml. of 95% ethyl alcohol, and 1.75 ml. (ca. 0.025 mole) of 37% aqueous formaldehyde was placed in a 25-ml. round-bottomed flask equipped with a thermometer. The mixture was agitated by means of a magnetic stirrer. One-tenth gram of sodium bicarbonate was added and the mixture stirred and warmed to 35°. Soon a clear solution was formed. Stirring was continued for one and one-half hours, at the end of which time the solution was still clear. The solution was then poured into 25 ml. of ice and water. A gummy solid soon began to separate. The flask was then refrigerated for forty-eight hours, during which time the gummy mass solidified. The solid was removed by filtration, yield 0.9 Gm. (64%). This was recrystallized from benzene to give a solid, m. p. 118–120°. On further recrystallization from benzene, the melting point was raised to 122°.

Anal. Calcd. for $C_{13}H_{13}Cl_2NO_3$: C, 51.67; H, 4.34. Found: C, 51.96; H, 4.41.

REFERENCES

- (1) Fuson, R. C., *Chem. Revs.*, **16**, 1(1935).
- (2) Burckhalter, J. H., and Johnson, S. H., *J. Am. Chem. Soc.*, **73**, 4835(1951).
- (3) Long, L. M., and Troutman, H. D., *ibid.*, **71**, 2469, 2473 (1949).
- (4) Robinson, R., and Foulds, R. P., *J. Chem. Soc.*, 103, 1768(1913).
- (5) Pain, D. L., and Slack, R., *ibid.*, 1951, 2529.
- (6) Hays, J. T., Hager, G. F., Engelmann, H. M., and Spurlin, H. M., *J. Am. Chem. Soc.*, **73**, 5369(1951).
- (7) Long, L. M., and Troutman, H. D., *ibid.*, **73**, 481 (1951).

Further Studies on *l*-Arterenol Tachyphylaxis in the Isolated *Venus mercenaria* Heart*

By TSUNEO FUJITA† and DAVID E. MANN, JR.‡

A TACHYPHYLACTIC RESPONSE to a pharmacological agent is characterized by a diminished response to successive doses of the same agent within a limited time interval. This phenomenon is inherent to a relatively small number of drugs, among which are renin (1), posterior pituitary extracts (2), atropine (3), and various sympathomimetic amines (4). According to Brown (5), there is general agreement that tachyphylaxis does not occur to the pressor response evoked by *l*-arterenol. However, Pless and Mann (6) observed a tachyphylactic response in the isolated *Venus mercenaria* heart upon successive administrations of *l*-arterenol.

These investigators noted that the inhibitory response to *l*-arterenol was decreasingly inhibited upon the first two administrations, with no response to the third. When a fresh heart was im-

mersed in sea water in which tachyphylaxis had been previously demonstrated, the heart failed to respond to the initial dose of *l*-arterenol. The tachyphylactic response was attributed to the presence of oxidation product(s) of *l*-arterenol since a pink discoloration of the sea water was noted coincident with the onset of tachyphylaxis. They also noted that pretreatment with varying concentrations of ephedrine sulfate altered the response to *l*-arterenol, and with sufficiently high concentrations, the response to *l*-arterenol was completely blocked.

Further investigation of the responses of the *Venus* heart to *l*-arterenol was undertaken to confirm these observations.

EXPERIMENTAL

The soft parts of the clam were exposed dorsally

TABLE I—PER CENT INHIBITION OF THE PREINJECTION AMPLITUDE OF THE *Venus* HEART AFTER THE ADMINISTRATION OF *l*-ARTERENOL CLASSIFIED ACCORDING TO TYPE OF RESPONSE

No Detn	Type I				No. Detn.	Type II			
	Concn. <i>l</i> -Arterenol					Concn. <i>l</i> -Arterenol			
	1×10^{-3}	2×10^{-3}	3×10^{-3}	4×10^{-3}		1×10^{-3}	2×10^{-3}	3×10^{-3}	4×10^{-3}
1	25	24	0	..	1	20	10	0	..
2	29	18	0	..	2	20	16	0	..
3	26	17	0	..	3	26	47	0	..
4	34	24	0	..	4	33	32	0	..
5	34	70	0	..	5	35	30	0	..
6	36	91	0	..	6	47	50	0	..
7	47	31	0	..	7	50	37	0	..
8	49	29	0	..	8	58	15	0	..
9	49	45	0	..	9	60	29	0	..
10	61	54	0	..	10	72	40	0	..
11	65	36	0	..	11	80	58	0	..
12	20	12	8	0	12	53	24	26	0
13	22	44	12	0	13	62	87	24	..
14	22	44	14	0	14	31	21	11	6
15	38	21	2	0	Type III				
16	50	40	38	0	1	17	15	0	..
17	54	39	23	0	2	50	23	0	..
18	78	24	30	0	3	24	34	40	0
19	29	9	14	..	4	32	22	21	0
20	22	24	8	..	5	20	24	11	..
21	19	8	8	..	6	28	20	19	..
22	67	17	10	..	7	26	35	7	27
23	50	44	23	26					
24	38	36	34	..					

* Received May 3, 1957, from Temple University School of Pharmacy, Philadelphia 40, Pa.
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by first cracking the shell with a gentle hammer blow, severing the adductor muscles, and removing the umbo and hinge of the valves. By careful dissection of the mantle and pericardium, the single median ventricle and one of the two laterally-disposed, thin-walled auricles were exposed. The auricle was severed after a threaded, size-zero fishhook was passed through the ventricle at the

auriculo-ventricular junction. The dorsal ventricular wall was cut antero-posteriorly and then completely severed from the intestine which passes through the ventricle. After severing the remaining auricle, the heart was attached to a chamber containing 40 ml. of sea water at room temperature (24–30°) and aerated with air. The thread was attached to a heart lever and recordings were made on a slow, smoked-drum, Livingston kymograph.

The figures tabulated in the tables represent the responses (in per cent inhibition) to *l*-arterenol which were determined by measuring the lowest amplitude within three minutes after each administration of *l*-arterenol, and comparing it with the amplitude recorded immediately preceding the administration of the drug.

Demonstration of Tachyphylaxis on the Isolated *Venus* Heart.—Three or four 0.4-ml. doses of *l*-arterenol (Levophed Bitartrate, 0.1% base, Winthrop Laboratories) were administered every six minutes to the bathing fluid without washing the heart, so that concentrations of 1×10^{-5} , 2×10^{-5} , 3×10^{-5} , and 4×10^{-5} of *l*-arterenol (as the base) were successively attained. A lack of response to the third or fourth dose of *l*-arterenol (3×10^{-5} or 4×10^{-5}) was indicative of tachyphylaxis.

In 45 determinations, 24 and 10 hearts were unresponsive at concentrations of 3×10^{-5} and 4×10^{-5} respectively. The remaining 11 hearts either were not completely inhibited or were markedly depressed as indicated by a negative inotropic and chronotropic response (Table I).

With the initial concentration of 1×10^{-5} of *l*-arterenol, a transient inhibition of amplitude of contraction was noted. The duration of inhibition varied, but generally recovered within three minutes. A transient increase in frequency as well as an increase in tone were noted, both of which were generally diminished with subsequent doses. However, the magnitude of inhibition varied over a wide range with each animal.

Responses to *l*-arterenol may be classified into three types, with the majority falling into the first two categories. After recovery from a transient inhibition, the amplitude returns essentially to normal in the first type (Fig. 1); the amplitude becomes greater than normal in the second type (Fig. 2); the third type is characterized by the failure of the heart to recover from the initial inhibition, and with the subsequent administration of *l*-arterenol, a greater degree of inhibition occurs.

These variations in responses may be attributed in part to the variable size and freshness of the clams.

The Effect of Pretreatment with Ephedrine Sulfate on the Demonstration of Tachyphylaxis.—After a normal recording was taken, varying quantities (0.13, 0.27, 0.40, and 0.50 milliliter) of 1% ephedrine sulfate were added to the bath to attain final concentrations (as the salt) of 3×10^{-5} , 7×10^{-5} , 1×10^{-4} , and 1.2×10^{-4} respectively. After a four-minute interval, the heart was challenged with three or four 0.4-ml. administrations of *l*-arterenol.

In these pretreated hearts, the degree of inhibition after each administration of *l*-arterenol was diminished when compared to the inhibition produced by *l*-arterenol alone. Tachyphylaxis was demonstrated upon the third or fourth dose of *l*-arterenol

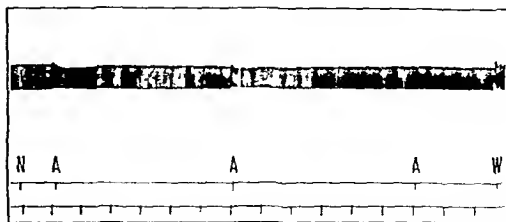


Fig. 1.—Demonstration of tachyphylaxis on the isolated *Venus mercenaria* heart with *l*-arterenol. Type 1. Legend: N—normal contraction; A—successive bath concentrations of *l*-arterenol as follows: 1×10^{-5} , 2×10^{-5} , 3×10^{-5} ; W—wash with fresh sea water. Time—in minutes.

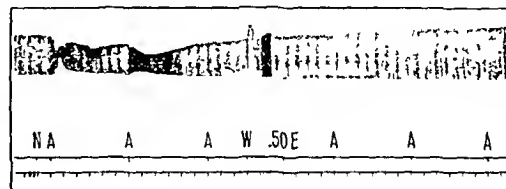


Fig. 2.—*l*-Arterenol-elicited responses of the *Venus* heart pretreated with ephedrine sulfate (1.2×10^{-4}). Legend: N—normal contraction; A—successive bath concentrations of *l*-arterenol: 1×10^{-5} , 2×10^{-5} , 3×10^{-5} , 1×10^{-5} , and 2×10^{-5} ; W—wash with fresh sea water; .50 E—ephedrine sulfate concentration (1.2×10^{-4}); Time—in minutes.

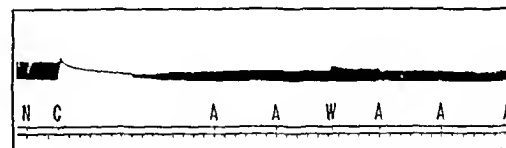


Fig. 3.—*l*-Arterenol responses of the isolated *Venus* heart bathed in previously oxidized sea water. Legend: N—normal contraction; C—change to previously oxidized sea water; A—successive bath concentrations of *l*-Arterenol: 1×10^{-5} , 2×10^{-5} ; W—wash with fresh sea water; Time—in minutes.

in hearts pretreated with 3×10^{-5} concentrations of ephedrine (5 determinations). The responses were varied in those pretreated with 7×10^{-5} concentrations (9 determinations) and 1×10^{-4} (16 determinations) concentrations of ephedrine sulfate, while with 1.2×10^{-4} (8 determinations), the response to *l*-arterenol was completely blocked (Fig. 2, Table II).

Although pretreatment with 3×10^{-5} of ephedrine sulfate had no effect on the production of tachyphylaxis, varied responses were noted in hearts pretreated with concentrations of 7×10^{-5} and 1×10^{-4} respectively. In eight cases, the heart was refractory to 1×10^{-5} of *l*-arterenol but responses occurred with higher concentrations; the heart was unresponsive to *l*-arterenol in five cases; tachyphylaxis was demonstrated in seven cases. How-

ever, pretreatment with 1.2×10^{-4} of ephedrine sulfate produced a complete blocking of response to *l*-arterenol.

TABLE II.—*l*-ARTERENOL-ELICITED RESPONSES^a OF THE *Venus* HEART PRETREATED WITH VARYING CONCENTRATIONS OF EPHEDRINE SULFATE, 1 PER CENT

No Detn	3×10^{-5} 1×10^{-4}	Ephedrine, Then 2×10^{-5} 2×10^{-4}	<i>l</i> -Arterenol Concn 3×10^{-5} 4×10^{-5}
1	12	0	0
2	15	27	0
3	17	19	0
4	18	28	11
5	100	17	26
No Detn	7×10^{-5} 1×10^{-4}	Ephedrine, Then 2×10^{-5} 2×10^{-4}	<i>l</i> -Arterenol Concn 3×10^{-5} 4×10^{-5}
1	0	0	0
2	0	20	0
3	0	36	0
4	0	9	17
5	12	6	0
6	16	14	0
7	35	13	0
8	62	16	0
9	32	23	22
No Detn	1×10^{-4} 1×10^{-3}	Ephedrine, Then 2×10^{-5} 2×10^{-4}	<i>l</i> -Arterenol Concn 3×10^{-5} 4×10^{-5}
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	10	0
6	0	23	0
7	0	10	11
8	0	27	15
9	0	41	63
10	9	0	0
11	29	0	0
12	23	31	0
13	36	40	0
14	53	8	0
15	14	7	7
16	52	32	6
No Detn	1.2×10^{-4} 1×10^{-3}	Ephedrine, Then 2×10^{-5} 2×10^{-4}	<i>l</i> -Arterenol Concn 3×10^{-5} 4×10^{-5}
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	7	7

^a Per cent inhibition of the preadministration amplitude after *l*-arterenol

The Effect of Previous Oxidation on the Demonstration of Tachyphylaxis.—To determine the effect of previous oxidation of the bathing fluid on the demonstration of tachyphylaxis, a fresh heart was suspended in sea water in which tachyphylaxis had been previously demonstrated on a second heart. After recovery from the inhibition caused by this change of bathing fluid, the *Venus* heart was challenged with three 0.4-ml. administrations of *l*-arterenol.

Of the 22 determinations recorded, no responses were observed with 1×10^{-5} concentrations of *l*-arterenol in seven cases; in six cases, no response was observed with 2×10^{-5} of *l*-arterenol; in two

cases, tachyphylaxis was not demonstrated with 3×10^{-5} concentrations of *l*-arterenol. In the remaining cases, the heart either failed to recover from the inhibition which occurred consistently with the change in bathing fluid or failed to recover to a sufficient degree to carry out the remaining procedures (Table III).

The normal heart is immediately arrested in systole upon changing to the bathing fluid in which tachyphylaxis had previously been demonstrated. During a variable time interval (averaging 7–9 minutes), the heart gradually relaxed until the contraction returned spontaneously, usually at a normal rate, and the amplitude progressively increased. When the contraction had attained a maximum, the heart became unresponsive to either the 1×10^{-5} or 2×10^{-5} concentration of *l*-arterenol (Fig. 3).

TABLE III.—*l*-ARTERENOL RESPONSES^a OF THE ISOLATED *Venus* HEART BATHED IN PREVIOUSLY OXIDIZED SEA WATER

No. Detn.	Concn of <i>l</i> -Arterenol—		
	1×10^{-4}	2×10^{-4}	3×10^{-4}
1	0	0	0
2	0	0	0
3	0	0	..
3	0	0	.
4	0	0	..
5	0	0	.
6	0	.	..
7	0	..	.
8	7	0	.
9	8	0	.
10	9	0	.
11	31	0	.
12	31	0	..
13	21	0	8
14	27	9	7
15	17	36	40
16–22	Insufficient recovery or no recovery from inhibition.		

^a Per cent inhibition of the preadministration amplitude following each 0.4 ml. dose of *l*-arterenol.

DISCUSSION

Throughout these experiments with *l*-arterenol, the pink discoloration referred to by Pless and Mann (6) was not observed, but by increasing the rate of aeration beyond the normal requirements, a pink discoloration was noted.

Although a discoloration was not observed in these experiments, the oxidation of *l*-arterenol must occur to some extent, due to the instability of *l*-arterenol in the presence of various chemical and physical factors which are present in this experimental procedure. However, since the inhibition caused by the change to the previously-oxidized solution resembles the response to a higher concentration of *l*-arterenol, this would indicate that the oxidation of *l*-arterenol had not occurred to any appreciable extent

In speculation, regarding the basis for *l*-arterenol tachyphylaxis, Welsh (7) postulated that 5-hydroxy-tryptamine may be the normal excitatory mediator

of the *Venus* heart for the responsiveness of the heart occurs to extremely low concentrations of this agent. Ergotoxine ethane sulfonate (8) is also stimulatory, while lysergie acid diethylamide (9) specifically blocks the action of 5-hydroxytryptamine. Since all of these compounds have an indole configuration in common, perhaps the *l*-arterenol may be oxidized to some indole or indole-like configuration at the receptor sites. The greater sensitivity of the postulated *l*-arterenol oxidation product(s) may overcome the inhibitory action of *l*-arterenol and with the subsequent addition of the latter, no further inhibitory response can be exerted; thereby eliciting tachyphylaxis. The oxidation product(s) could be formed in the bathing fluid, but in view of the prolonged inhibition of rhythm which occurs with the change of bathing fluid, this seems unlikely according to this postulation.

SUMMARY

Although great variations in the response to repeated administrations of *l*-arterenol have been observed, tachyphylaxis was demonstrated in the

isolated *Venus mercenaria* heart. The response to *l*-arterenol was progressively altered by pretreatment with increasing concentrations of ephedrine sulfate, while sufficiently high concentrations completely blocked the typical *l*-arterenol response. The fresh heart was generally refractory to either 1×10^{-5} or 2×10^{-5} concentrations of *l*-arterenol when suspended in bathing fluid in which tachyphylaxis had been previously demonstrated.

REFERENCES

- (1) Goldblatt, H., Lamform, H., Haas, E., *Am. J. Physiol.*, **175**, 75(1953).
- (2) Geiling, E. M. K., *Physiol. Revs.*, **6**, 62(1926).
- (3) Grey, G., SeEVERS, M., *J. Pharmacol. Exptl. Therp.*, **113**, 319(1955).
- (4) Winder, C. V., Anderson, M. M., Parke, H. C., *J. Pharmacol. Exptl. Therp.*, **93**, 63(1948).
- (5) Brown, R. V., *ibid.*, **105**, 139(1952).
- (6) Pless, H. H., Personal Communication.
- (7) Welsh, J. H., *Fed. Proc.*, **13**, 162(1954).
- (8) Welsh, J. H., *Arch. exptl. Pathol. Pharmacol.*, **219**, 23(1953).
- (9) Welsh, J. H., *Nature*, **173**, 955(1954).

Dextran Localization and Retention in Normal and X-Ray Irradiated Rabbits*

By LAWRENCE E. DETRICK, HARVEY C. UPHAM, DOROTHY HIGHBY,
and THOMAS J. HALEY

Intravenous saline or dextran had no influence upon the effects produced by 500 r acute whole body x-irradiation in rabbits. The irradiation caused a post-irradiation shock-like syndrome in rabbits and minor histopathological changes in the liver. Neither saline nor dextran affected the fatal termination of the syndrome. In rabbits, 500 r x-irradiation was not a noxious stress of sufficient magnitude to impair metabolic or excretory functions of organs and alter dextran storage in tissues examined sixty days later. When examined sixty days post-irradiation, there was no significant difference in tissue storage or distribution of dextran between irradiated and nonirradiated animals. The intensity of dextran storage within rabbit organs was directly proportional to dosage and inversely proportional to the time after injection in those organs examined prior to the sixty day sacrifice interval. Irradiation did not affect storage either qualitatively or quantitatively.

A PARENTERAL SOLUTION of the polysaccharide dextran has been under intensive investigation both clinically (1-3) and experimentally (4-6), because of its potentiality as a safe, effective, and readily available plasma volume

expander. Dextran has been recommended for the treatment of the mass casualties which might occur during a disaster (7). Information on the toxicity and tissue retention of intravenously injected dextran following acute whole body irradiation is lacking. Such information is necessary because any condition which damages the metabolic and excretory organs may lead to tissue storage of injected material (8). The present investigation has studied the toxicity, tissue distribution, and retention of intravenously

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injected dextran¹ solution in normal and irradiated rabbits.

EXPERIMENTAL

Female Dutch strain rabbits weighing 1.4 to 1.6 Kg. were used after implanting an indwelling catheter according to the method of Detrick and Rhodes (9). Groups of eight animals each were used for the single injection series; and groups of 12 animals each were used for the multiple injection series (see Table I for details). Each injection,

TABLE I.—DEXTRAN INJECTION IN RABBITS

Group No. ^a	Post r Injection Time	No. of Injections	Solution Injected	Amount of Dextran	
				ml.	Gm.
1	1 hr.	1	Dextran	75	4.5
2	1 day	1	Dextran	75	4.5
3	3 day	1	Dextran	75	4.5
4	7 day	1	Dextran	75	4.5
5	11 day	1	Dextran	75	4.5
6	1 hr.	1	Saline	75	0
7	3 day	1	Saline	75	0
8	0	0	Control	0	0
9	1 day ^b	1	Dextran	75	4.5
10	1 day ^b	1	Saline	75	0
11	1 day	9	Dextran	75	40.5
12	1 day	9	Saline	675	0
13	1 day ^b	9	Dextran	675	40.5
14	1 day ^b	9	Saline	675	0
15	0	0	Control	0	0

^a Groups 1-10 contained 8 animals each. Groups 11-15 contained 12 animals each
^b Non irradiated controls.

These latter animals were subjected to a 72-hr. food deprivation period prior to sacrifice to reduce body glycogen stores and thus reduce false positive staining reactions. Tissue specimens (Table II) were removed, fixed in neutral formalin, and stained with hematoxylin and eosin. Contiguous slices of the above specimens were also fixed in cold absolute alcohol and serial tissue sections were differentially stained by the alcoholic periodic acid-aqueous Schiff's method for dextran (4). Five hundred r acute whole body x-irradiation was administered to the animals from above and below with two 250 KVP Picker Industrial Units operating simultaneously. Technical factors were: 250 KVP; FOD 55 cm.; filters—Cu 0.21 mm. inherent and 0.5 mm. parabolic and 1 mm. Al; size of field—whole body; rate—50.25 r/min. measured in air with a Victoreen Thimble r-meter.

RESULTS

General Effects of Infusion and Irradiation.—After sixty days, the saline control groups (Table I) showed no changes which could be attributed to the saline infusions. However, the nonirradiated animals showed varying degrees of PAS staining which has been attributed to glycogen, basement membrane, mucin, colloid, and hyaline materials (1). Such naturally occurring materials were observed in all tissues examined and did not appear to increase following irradiation. The pre-sacrifice fasting of the animals appeared to decrease this staining reaction, thus increasing the probability that the remaining material found in the tissues of the dextran

TABLE II.—INTENSITY OF DEXTRAN STORAGE IN ORGANS OF IRRADIATED AND NONIRRADIATED RABBITS^a

No. of Dextran Injections Radiation Survival in Days	Single ^b					Nine ^b		
	500 r			Sacrificed 60	None Sacrificed 60	500		None Sacrificed 60
	8	Died 15	32			Died 19	Sacrificed 60	
Liver	4	3	2	0-3	1-2	5	4	4
Kidney	4	3	1	0-1	0-1	5	3	3-4
Adrenal	1	1	0	0	0-1	5	2-3	3-4
Spleen	3	1	0	0	0	5	2-3	3
Lymph node	.	.	.	0-1	0	4	2-3	2-3
Bone marrow	4	2	1	0-1	0-1	5	3-4	3
Lung	2	2	2	0-1	0	4	1	1
Pancreas	3	2	2	0-1	0-1	5	1-3	1-3
Small bowel	1	4	1	0-1	0-1	2	0-1	1
Large bowel	.	.	.	0-1	0-1	4	1-2	2
Muscle	2	1	1	0	0	3	1	1
Heart	3	3	1	1-2	0-1	4	1	2
No. of animals		10		30	8	1	11	12

^a Intensity of dextran storage: 1—minimal; 2—slight; 3—moderate; 4—abundant; 5—intensive.
None-dextran injected groups omitted: Number of animals, single (S), multiple (M). Irradiated saline S (16), M (12); Nonirradiated saline S (8), M (12); irradiated S (8); M (11).

saline or dextran, was of a total volume of 75-ml. given at a rate of 25 ml./hr. This clinical dextran contained 5-10% of material with average molecular weights as high as 200,000 and as low as 25,000, with the balance having an average molecular weight of 70,000. Necropsies were performed on all animals that died during the course of the experiments and on the survivors sixty days post-irradi-

treated animals was actually dextran.
The 500 r whole body irradiation produced a 13% mortality in sixty days. Post-irradiation shock-like deaths (5-7 hours PR) were ushered in by a short period of restlessness, inactivity, fall in body temperature, lack of response to mild stimuli, and terminal convulsions. Intravenous injections of either dextran or saline solution administered during the development of the shock-like syndrome in no way influenced the eventual fatal outcome. Pneumonitis, uncomplicated by dextran injections, was the

¹ The authors wish to thank Dr. Walter E. Ward of Cutter Laboratories for the clinical dextran solutions (Lot #S293-B) used.

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Female Dutch strain rabbits weighing 1.4 to 1.6 Kg. were used after implanting an indwelling catheter according to the method of Dietrick and Rhodes (9). Groups of eight animals each were used for the single injection series; and groups of 12 animals each were used for the multiple injection series (see Table I for details). Each injection,

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Group	No. ^a Post r Infection Time	No of Injections	Solution Injected	Amount of Dextran ml
1	1 hr.	1	Dextran	4.5
2	1 day	1	Dextran	4.5
3	3 day	1	Dextran	4.5
4	7 day	1	Dextran	4.5
5	11 day	1	Dextran	4.5
6	1 hr.	1	Saline	0
7	3 day	1	Saline	0
8	0	0	Control	0
9	1 day ^b	1	Dextran	4.5
10	1 day ^b	1	Saline	0
11	1 day	9	Dextran	40.5
12	1 day	9	Saline	0
13	1 day ^b	9	Dextran	40.5
14	1 day ^b	9	Saline	0
15	0	0	Control	0

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RESULTS

ation. These latter animals were subjected to a 72-hr. food deprivation period prior to sacrifice to reduce body glycogen stores and thus reduce false positive staining reactions. Tissue specimens (Table II) were removed, fixed in neutral formalin, and stained with hematoxylin and eosin. Contiguous slices of the above specimens were also fixed in cold absolute alcohol and serial tissue sections were differentially stained by the alcoholic periodic acid-alkaline Schiff's method for dextran (4). Five hundred μ acute whole body x-irradiation was administered to the animals from above and below with two 250 KVp Picker Industrial Units operating simultaneously. Technical factors were: 250 KVp; FOD 55 cm.; filters—Cu 0.21 mm. inherent and 0.5 mm. paraffolic and 1 mm. Al; size of field—whole body; rate—50.25 r/min. measured in air with a Victoreen Thimble-r-meter.

TABLE II—INTENSITY OF DEXTRAN STORAGE IN ORGANS OF IRRADIATED AND NONIRRADIATED RABBITS*

[illegible]

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5	11 day	1	Dextran	75	4.5
6	1 hr.	1	Saline	75	0
7	3 day	1	Saline	75	0
8	0	0	Control	0	0
9	1 day ^b	1	Dextran	75	4.5
10	1 day ^b	1	Saline	75	0
11	1 day	9	Dextran	75	40.5
12	1 day	9	Saline	675	0
13	1 day ^b	9	Dextran	675	40.5
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Liver	4	3	2	0-3	1-2	5	4		4	
Kidney	4	3	1	0-1	0-1	5	3		3-4	
Adrenal	1	1	0	0	0-1	5	2-3		3-4	
Spleen	3	1	0	0	0	5	2-3		3	
Lymph node	.	.	.	0-1	0	4	2-3		2-3	
Bone marrow	4	2	1	0-1	0-1	5	3-4		3	
Lung	2	2	2	0-1	0	4	1		1	
Pancreas	3	2	2	0-1	0-1	5	1-3		1-3	
Small bowel	1	4	1	0-1	0-1	2	0-1		1	
Large bowel	.	.	.	0-1	0-1	4	1-2		2	
Muscle	2	1	1	0	0	3	1		1	
Heart	3	3	1	1-2	0-1	4	1		2	
No. of animals		10		30	8	1	11		12	

^a Intensity of dextran storage: 1—minimal; 2—slight; 3—moderate; 4—abundant; 5—intensive.
Non-dextran injected groups omitted: Number of animals, single (S), multiple (M). Irradiated saline S (16), M (12); Nonirradiated saline S (8), M (12); irradiated S (8); M (11).

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No. of Dextran Injections	Radiation Survival in Days	Liver	Kidney	Adrenal	Spleen	Lymph node	Bone marrow	Lung	Pancreas	Small bowel	Large bowel	Muscle	Heart	No. of animals
8	Died 15	4	4	1	3	4	2	2	2	1	2	3	3	10
7	32	2	1	0	0	1	0-1	0-1	0-1	1	0-1	0	1-2	30
6	60	0-3	0-1	0	0	0-1	0	0-1	0-1	0-1	0-1	0	0	8
None	Sacri ficed 60	1-2	0-1	0-1	0	0	0-1	0	0-1	0-1	0-1	0	0-1	12
500	500	Died 19	5	5	5	5	5	5	5	2	4	3	4	1
Nine	Sacri- ficed 60	4	3	2-3	2-3	2-3	2-3	3-4	1	0-1	1-2	1	1	11
None	Sacri- ficed 60	4	3-4	3-4	3	3	2-3	3	1	0-1	1	1	1	12

The 500 r whole body irradiation produced a 13% mortality in six days. Post-irradiation shock-like deaths (5-7 hours PR) were ushered in by a short period of restlessness, inactivity, fall in body temperature, lack of response to mild stimuli, and terminal convulsions. Intravenous injections of either dextran or saline solution administered during the development of the shock-like syndrome in no way influenced the eventual fatal outcome. Pneumothorax, unoperated by dextran injections, was the

saline or dextrose solution, was of a total volume of 70 ml. This clinical dextrose contained 5–10% of material with average molecular weights as high as 200,000 and as low as 25,000, of which 2,000 experiments were performed on all animals that died during the course of the experiment and on the survivors sixty days post-irradiation.

(11) X' (8) S dehydrated saline X' (7) N (8) S saline dehydrated

The authors wish to thank Dr. Walter L. Ward of Cutter Laboratories for the clinical dextran solutions (Lot #58293-B) used.

TABLE I.—Dextran Injection in Rabbits

Group	No. of Injections	Post Infection Time	No. of Injections	Solution Injected	Amount of Dextran ml	Group
1	1 hr	1 day	1	Dextran	75	4 5
2	1 hr	1 day	1	Dextran	75	4 5
3	3 days	7 days	1	Dextran	75	4 5
4	7 days	11 days	1	Dextran	75	4 5
5	11 days	1 hr	1	Dextran	75	4 5
6	1 hr	3 days	1	Saline	75	0
7	3 days	0	0	Saline	75	0
8	0	1 day	1	Control	0	0
9	1 day	1 day	1	Dextran	75	4 5
10	1 day	1 day	1	Saline	75	0
11	1 day	1 day	9	Dextran	675	0
12	1 day	1 day	9	Saline	675	0
13	1 day	1 day	9	Dextran	675	0
14	1 day	1 day	9	Saline	675	0
15	0	0	0	Control	0	0

Female Dutch strain rabbits weighing 1.5 to 1.6 Kg. were used after implanting an indwelling catheter according to the method of Detrick and Rhodes (9). Groups of eight animals each were used for the single injection series; and groups of 12 animals each were used for the multiple injection series (see Table I for details). Each injection,

EXPERIMENTAL

injected devtran¹ solution in normal and irradiated rabbits

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EXPERIMENTAL

Female Dutch strain rabbits weighing 1.4 to 1.6 Kg. were used after implanting an indwelling catheter according to the method of Detrick and Rhodes (9). Groups of eight animals each were used for the single injection series; and groups of 12 animals each were used for the multiple injection series (see Table I for details). Each injection,

TABLE I.—DEXTRAN INJECTION IN RABBITS

Group No. ^a	Post r Injection Time	No. of Injections	Solution Injected	Amount of Dextran ml.	Gm.
1	1 hr.	1	Dextran	75	4.5
2	1 day	1	Dextran	75	4.5
3	3 day	1	Dextran	75	4.5
4	7 day	1	Dextran	75	4.5
5	11 day	1	Dextran	75	4.5
6	1 hr.	1	Saline	75	0
7	3 day	1	Saline	75	0
8	0	0	Control	0	0
9	1 day ^b	1	Dextran	75	4.5
10	1 day ^b	1	Saline	75	0
11	1 day	9	Dextran	75	40.5
12	1 day	9	Saline	675	0
13	1 day ^b	9	Dextran	675	40.5
14	1 day ^b	9	Saline	675	0
15	0	0	Control	0	0

^a Groups 1-10 contained 8 animals each. Groups 11-15 contained 12 animals each.

^b Non irradiated controls.

These latter animals were subjected to a 72-hr. food deprivation period prior to sacrifice to reduce body glycogen stores and thus reduce false positive staining reactions. Tissue specimens (Table II) were removed, fixed in neutral formalin, and stained with hematoxylin and eosin. Contiguous slices of the above specimens were also fixed in cold absolute alcohol and serial tissue sections were differentially stained by the alcoholic periodic acid-aqueous Schiff's method for dextran (4). Five hundred r acute whole body x-irradiation was administered to the animals from above and below with two 250 KVP Pieker Industrial Units operating simultaneously. Technical factors were: 250 KVP; FOD 55 cm.; filters—Cu 0.21 mm. inherent and 0.5 mm. parabolic and 1 mm. Al; size of field—whole body; rate—50.25 r/min. measured in air with a Victoreen Thimble r-meter.

RESULTS

General Effects of Infusion and Irradiation.—After sixty days, the saline control groups (Table I) showed no changes which could be attributed to the saline infusions. However, the nonirradiated animals showed varying degrees of PAS staining which has been attributed to glycogen, basement membrane, mucin, colloid, and hyaline materials (1). Such naturally occurring materials were observed in all tissues examined and did not appear to increase following irradiation. The pre-sacrifice staining of the animals appeared to decrease this staining reaction, thus increasing the probability that the remaining material found in the tissues of the dextran

TABLE II.—INTENSITY OF DEXTRAN STORAGE IN ORGANS OF IRRADIATED AND NONIRRADIATED RABBITS^a

No. of Dextran Injections Radiation Survival in Days	Single ^b				Nine ^b			
	500 r			None Sacrificed 60	500 r			None Sacrificed 60
	8	Died 15	32		Died 19	Sacrificed 00		
Liver	4	3	2	0-3	1-2	5	4	4
Kidney	4	3	1	0-1	0-1	5	3	3-4
Adrenal	1	1	0	0	0-1	5	2-3	3-4
Spleen	3	1	0	0	0	5	2-3	3
Lymph node				0-1	0	4	2-3	2-3
Bone marrow	4	2	1	0-1	0-1	5	3-4	3
Lung	2	2	2	0-1	0	4	1	1
Pancreas	3	2	2	0-1	0-1	5	1-3	1-3
Small bowel	1	4	1	0-1	0-1	2	0-1	1
Large bowel				0-1	0-1	4	1-2	2
Muscle	2	1	1	0	0	3	1	1
Heart	3	3	1	1-2	0-1	4	1	2
No. of animals		10		30	8	1	11	12

^a Intensity of dextran storage: 1—minimal, 2—slight, 3—moderate, 4—abundant, 5—intensive.

^b Non dextran injected groups omitted. Number of animals, single (S), multiple (M). Irradiated saline S (16), M (12); Nonirradiated saline S (8), M (12), irradiated S (8), M (11)

saline or dextran, was of a total volume of 75-ml. given at a rate of 25 ml./hr. This clinical dextran contained 5-10% of material with average molecular weights as high as 200,000 and as low as 25,000, with the balance having an average molecular weight of 70,000. Necropsies were performed on all animals that died during the course of the experiments and on the survivors sixty days post-irradiation.

¹ The authors wish to thank Dr. Walter E. Ward of Cutter Laboratories for the clinical dextran solutions (Lot #58293-B) used.

treated animals was actually dextran.

The 500 r whole body irradiation produced a 13% mortality in sixty days. Post-irradiation shock-like deaths (5-7 hours PR) were ushered in by a short period of restlessness, inactivity, fall in body temperature, lack of response to mild stimuli, and terminal convulsions. Intravenous injections of either dextran or saline solution administered during the development of the shock-like syndrome in no way influenced the eventual fatal outcome. Pneumonitis, uncomplicated by dextran injections, was the

injected dextran¹ solution in normal and irradiated rabbits.

EXPERIMENTAL

Female Dutch strain rabbits weighing 1.4 to 1.6 Kg. were used after implanting an indwelling catheter according to the method of Detrick and Rhodes (9). Groups of eight animals each were used for the single injection series; and groups of 12 animals each were used for the multiple injection series (see Table I for details). Each injection,

TABLE I.—DEXTRAN INJECTION IN RABBITS

Group	No. of rabbits	Post injection time	No. of injections	Solution injected	Amount of dextran, ml.	Gm.
1	1	1 hr.	1	Dextran	75	4.5
2	1	1 day	1	Dextran	75	4.5
3	3	3 day	1	Dextran	75	4.5
4	5	7 day	1	Dextran	75	4.5
5	5	11 day	1	Dextran	75	4.5
6	6	1 hr.	1	Saline	75	0
7	8	3 day	0	Saline	75	0
8	0	Control	0	Control	0	0
9	1 day ^b	Dextran	1	Dextran	75	4.5
10	1 day ^b	Saline	1	Saline	75	0
11	1 day	Dextran	9	Dextran	75	40.5
12	1 day	Saline	9	Saline	675	0
13	1 day ^b	Dextran	9	Dextran	675	40.5
14	1 day ^b	Saline	9	Saline	675	0
15	0	Control	0	Control	0	0

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No. of Dextran injections	No. of animals	Liver	Kidney	Adrenal	Spleen	Lymph node	Bone marrow	Lung	Pancreas	Small bowel	Large bowel	Muscle	Heart
1	1	4	4	1	3	3	4	2	2	1	2	1	1
2	1	4	3	1	3	2	2	2	2	4	1	1	1
3	3	3	3	1	1	2	2	2	2	2	1	1	1
4	3	3	3	1	1	2	2	2	2	2	1	1	1
5	3	3	3	1	1	2	2	2	2	2	1	1	1
6	3	3	3	1	1	2	2	2	2	2	1	1	1
7	3	3	3	1	1	2	2	2	2	2	1	1	1
8	3	3	3	1	1	2	2	2	2	2	1	1	1
9	3	3	3	1	1	2	2	2	2	2	1	1	1
10	3	3	3	1	1	2	2	2	2	2	1	1	1
11	3	3	3	1	1	2	2	2	2	2	1	1	1
12	3	3	3	1	1	2	2	2	2	2	1	1	1
13	3	3	3	1	1	2	2	2	2	2	1	1	1
14	3	3	3	1	1	2	2	2	2	2	1	1	1
15	3	3	3	1	1	2	2	2	2	2	1	1	1

^a Intensity of dextran storage: 1—minimal; 2—slight; 3—moderate; 4—abundant; 5—intensive.

^b Non-dextran injected groups omitted: Number of animals, single (S), multiple (M). Irradiated saline S (10), M (12); Nonirradiated saline S (8), M (12); irradiated S (8), M (11).

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Kidney.—A heavier tissue deposition and a more generalized tissue involvement characterized storage phenomena within the kidney. Dextran was observed in all glomeruli varying in size from free granules to large masses of blue-black colored material. The number of localized solid blue tubular cells and casts was increased. Fine blue granules occurred in the majority of tubular cells and there was a much more prominent involvement of the interlobular reticulo-endothelial cells.

Adrenal.—Dextran deposition occurred in histiocytes of the surrounding fat, in and beneath the capsule. Cells of the zona glomerulosa and outer fasciculata were nearly solid blue, tapering off as discrete granular deposition in the inner fasciculata, reticularis, and medullary zones. Interstitial histiocytes of the entire organ were involved.

Spleen.—The spleen showed less shrinkage, a characteristic of recovery from irradiation. The follicles were larger and the capsule, trabeculae, and hemosiderin deposits less prominent. There was massive infiltration of free dextran granules in the red pulp. The white pulp was clear except for an occasional filled histiocyte in or about blood vessels. Free granules lined the sinusoids.

Lymph Nodes.—Only a delicate deposit of fine granular dextran was seen within reticulum cells and lining the sinusoids.

Bone Marrow.—Free dextran granules lightly infiltrated the marrow tissue and were present within blood vessels and filled the phagocytes.

Pancreas.—Dextran was stored as massive blue, triangular, extracellular infiltrations of free granules within interlobular connective tissues and as a diffuse granular infiltration in the cytoplasm of acinar cells, but not in the islet cells. The macrophages were filled and free granules were present within blood vessels.

Small and Large Bowel.—Blue staining material in histiocytes of the serosal coat, between muscle layers, and about blood vessels of the lamina propria, villi, and intestinal folds was observed.

Striated Muscle.—Storage did not occur within muscle fibers, but free granules were present in the connective tissue. Histiocytes in the connective tissue and about the blood vessels stained dark blue.

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DISCUSSION

Our findings agree with observations of others who found parenteral dextran a safe plasma volume expander for rabbits (6, 10). Single or multiple intravenous injections were uneventful; infiltration of free granules into the tissues, storage in parenchymal or phagocytic cells incited no histological reactions; observations to the contrary notwithstanding (11). An infusion of dextran was a safe, although ineffective, therapeutic procedure even when given during the developmental period of the irreversible post-irradiation shock. These findings indicate that dextran is much safer than dextrin, oxypolygelatin or PVP under the same experimental conditions (12-15).

Dextran tissue retention has been detected by histochemical, serological, and chemical methods (4, 6, 11). The alcoholic periodic acid-aqueous differential staining procedure was used successfully in dextran identification in the human (1), mouse (4), dog (8), and rabbit (16). Normal tissues contain no PAS-positive, water soluble material, but do show "naturally occurring" water insoluble materials such as glycogen, basement membrane, hyaline, colloid, and mucin (1). We observed similar staining reactions in control animals. Such staining reactions were not increased by irradiation. Irradiated dextran injected rabbits that died early showed abundant red hyaline casts throughout the kidney. Similar red casts have been reported in kidney tissue of both battle casualties (1) and burned mice (17). Irradiated splenic tissue also showed PAS-positive staining cells, but a generalized plasma cell cytotoxicity was not seen in the rabbits. However, aggregates of PAS-positive plasma cells have been observed in rabbit spleens following stress of hyperimmunization (18), and plasma cell cytotoxicity was a consistent finding in atomic bomb casualty tissues (19).

Intravenously injected dextran produced retention in parenchymal cells of the liver, kidney, adrenal and pancreas, and in reticulo-endothelial cells of all organs examined including the spleen, lymph nodes, bone marrow, lung, bowel, and muscle. The intensity of deposition was proportional to the amount given and inversely proportional to the post-injection time at which the tissues were examined. Dextran solutions were not tissue irritants in moderate amounts. The above findings agree with observations of others (1-6, 17, 20). Bull, *et al.* (6), pointed out that the lower molecular weight fractions of dextran were rapidly excreted whereas the higher fractions were retained in the body. Thus our observations of tissue deposition are probably related to retention of the higher molecular weight fractions in the dextran we used. Gray (21) using C^{14} -labeled dextran showed that eventually even the retained fraction is eliminated from the body. The observation of decreased tissue retention (Table II) confirms Gray (21).

The irradiation, itself, produced minor changes in the liver, kidney, bone marrow, spleen, lymphoid tissue, and lung. This agrees with the observations of Hagen, *et al.* (22).

REFERENCES

- (1) Vickery, A. L., *Am. J. Pathol.*, **32**, 161(1956).
- (2) Jaenike, J. R., and Waterhouse, C., *Circulation*, **11**, 1(1955).
- (3) Norlander, O., Troell, L., and Aberg, B., *Acta Chir. Scand.*, **109**, 1(1955).
- (4) Mowry, R. W., and Millican, R. C., *Am. J. Pathol.*, **29**, 523(1953).
- (5) Friberg, U., Graf, W., and Aberg, B., *Acta Pharmacol. Toxicol.*, **9**, 220(1953).
- (6) Bull, J. P., Ricketts, C., Squire, J. R., Maycock, W. d'a., Spooner, S. J. L., Mollinson, P. L., and Patterson, J. C., *Lancet*, **1**, 134(1949).
- (7) Ampacher, W. H., and Gray, I., *Ann. N. Y. Acad. Sci.*, **55**, 526(1952).
- (8) Pirani, C. L., Juster, R., Ingraham, R. J., Froeh, H. F., Bell, J. H., and Consolazio, C. R., Medical Nutrition Laboratory, U. S. Army, Fitzsimons Army Hospital Rept. 124(1954).
- (9) Detrick, L. E., and Rhodes, B. J., *THIS JOURNAL*, **41**, 238(1952).
- (10) Ingelman, B., *Uppsala Lakareförenings Förhandlingar*, **54**, 107(1949).
- (11) Persson, B. H., *Acta Soc. Med. Uppsala*, **57**, 421(1952).
- (12) Detrick, L. E., Lewis, A. E., Highby, D., Rhodes, B., and Haley, T. J., *THIS JOURNAL*, **41**, 614(1952).

- (13) Detrick, L. E., Debley, V., and Haley, T. J., *ibid.*, 42, 623(1953).
 (14) Detrick, L. E., Debley, V., and Haley, T. J., *ibid.*, 43, 449(1954).
 (15) Upham, H. C., Lovell, W., Detrick, L. E., Highby, D. H., Debley, V., and Haley, T. J., *Arch. intern. Pharmacodyn.*, 104, 151(1956).
 (16) Friberg, U., Graf, W., and Aberg, B., *Scand. J. Clin. & Lab. Invest.*, 3, 221(1951).
 (17) Mowry, R. W., Longley, J. B., and Millican, R. C.,

- J. Lab. Clin. Med.*, 39, 211(1952).
 (18) Teilum, G., *Am. J. Pathol.*, 32, 945(1956).
 (19) Liebow, A. A., Warren, S., and De Coursey, E., *ibid.*, 25, 853(1949).
 (20) Hartman, F. W., and Behrmann, Y. G., *J. Am. Med. Assoc.*, 152, 1116(1953).
 (21) Grey, I., *Am. J. Physiol.*, 174, 462(1953).
 (22) Hagen, C. W., Jr., Jacobson, L. O., Murray, R., and Lear, P., *U. S. Atomic Energy Comm.*, MDDC-999, June 4, 1947.

The Ontogenesis of Gramine in Barley Seedlings*

By V. E. TYLER, Jr.

A sensitive paper chromatographic technique was developed for the separation and identification of the basic nitrogenous compounds in barley seedlings. This method was utilized to follow the ontogenesis of gramine in normal and etiolated plants. It was established that gramine does not exist in the ungerminated seed, the coleoptiles, or roots of Charlottetown No. 80 barley, but is present in the leaf on the third day following germination and remains there in detectable quantities for at least fifty days. No qualitative differences in gramine content were observed between the normal and etiolated seedlings. The significance of these results with reference to seemingly conflicting previous reports is discussed.

GRAMINE, AN ALKALOID isolated from certain varieties of barley, *Hordeum vulgare* L., and also from an Asiatic reed, *Arundo donax* L., has been characterized chemically as 3-(dimethylaminomethyl)-indole (1). Employing a photometric method of determination, Euler, *et al.* (2, 3), established that of 18 varieties of barley seedlings investigated, five contained gramine. In these plants the alkaloid was not present in the coleoptile, but was detected in the roots and leaves, increasing in concentration in the latter organs from the base to the apex. It was further established that the total gramine content of the plant remains constant from the fourth to the tenth day following germination, but no alkaloid could be detected after one month.

More recently, Marion, *et al.*, in studies on the biogenesis of alkaloids, fed tryptophan labeled with C^{14} in the β position to germinating barley seedlings (variety Charlottetown No. 80) and were able to isolate radioactive gramine from the leaves, indicating that tryptophan serves as a precursor to gramine (4). Radioautographs of such barley leaves indicated that the tryptophan was first concentrated near the base of the leaves and then reappeared as gramine in the leaf tips

(5). A further study (6), employing a mixture of tryptophans labeled in the 2 and β positions, verified that the conversion of tryptophan to gramine takes place without cleavage of the indole-alanine linkage.

Because gramine is a derivative of indole, a structure which is of considerable physiological interest, it appeared worth while to obtain more detailed information regarding the ontogenesis of the alkaloid, especially with respect to its reported occurrence in the root as well as its ultimate disappearance from the leaf.

EXPERIMENTAL

Chromatographic Identification of Gramine.—Before proceeding with the ontogenetic phase of the investigation, it was necessary to develop a sensitive process for the separation and identification of the basic nitrogenous compounds occurring in barley seedlings; specifically choline, gramine, and hordenine. The paper chromatographic method appeared well suited for this purpose, but gramine and hordenine proved to be extremely difficult to separate completely, and a large number of the more conventional organic solvent systems were employed without success. Good resolution was finally achieved using an aqueous solution of an electrolyte as suggested by Resplandy (7).

Mixtures of the bases were spotted on 1×22.5 -in. strips of Whatman No. 1 filter paper and subjected to ascending formation with a 10% aqueous solution of ammonium sulfate for about five hours. A more rapid and equally satisfactory separation was achieved employing the circular formation method

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- (3) Norlander, O., Troell, L., and Aberg, B., *Acta Chir. Scand.*, **109**, 1(1955).
- (4) Mowry, R. W., and Millican, R. C., *Am. J. Pathol.*, **29**, 523(1952).
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- (7) Ampsacher, W. H., and Gray, I., *Ann. N. Y. Acad. Sci.*, **55**, 526(1952).
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- (9) Detrick, L. E., and Rhodes, B. J., *THIS JOURNAL*, **41**, 238(1952).
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- (11) Persson, B. H., *Acta Soc. Med. Uppsala*, **57**, 421(1952).
- (12) Detrick, L. E., Lewis, A. E., Highby, D., Rhodes, B., and Haley, T. J., *THIS JOURNAL*, **41**, 614(1952).

- (13) Detrick, L. E., Debley, V., and Haley, T. J., *ibid.*, 42, 623(1953).
(14) Detrick, L. E., Debley, V., and Haley, T. J., *ibid.*, 43, 449(1954).
(15) Upham, H. C., Lovell, W., Detrick, L. E., Highby, D. H., Debley, V., and Haley, T. J., *Arch. intern. Pharmacodyn.*, 104, 151(1956).
(16) Friberg, U., Graf, W., and Aherg, B., *Scand. J. Clin. & Lab. Invest.*, 3, 221(1951).
(17) Mowry, R. W., Longley, J. B., and Millican, R. C., *J. Lab. Clin. Med.*, 39, 211(1952).
(18) Tellum, G., *Am. J. Pathol.*, 32, 945(1956).
(19) Liebow, A. A., Warren, S., and De Coursey, E., *ibid.*, 25, 853(1949).
(20) Hartman, F. W., and Behrmann, Y. G., *J. Am. Med. Assoc.*, 152, 1116(1953).
(21) Grey, I., *Am. J. Physiol.*, 174, 462(1953).
(22) Hagen, C. W., Jr., Jacobson, L. O., Murray, R., and Lear, P., *U. S. Atomic Energy Comm.*, MDDC-999, June 4, 1947.

The Ontogenesis of Gramine in Barley Seedlings*

By V. E. TYLER, Jr.

A sensitive paper chromatographic technique was developed for the separation and identification of the basic nitrogenous compounds in barley seedlings. This method was utilized to follow the ontogenesis of gramine in normal and etiolated plants. It was established that gramine does not exist in the ungerminated seed, the coleoptiles, or roots of Charlottetown No. 80 barley, but is present in the leaf on the third day following germination and remains there in detectable quantities for at least fifty days. No qualitative differences in gramine content were observed between the normal and etiolated seedlings. The significance of these results with reference to seemingly conflicting previous reports is discussed.

GRAMINE, AN ALKALOID isolated from certain varieties of barley, *Hordeum vulgare* L., and also from an Asiatic reed, *Arundo donax* L., has been characterized chemically as 3-(dimethylaminomethyl)-indole (1). Employing a photometric method of determination, Euler, *et al.* (2, 3), established that of 18 varieties of barley seedlings investigated, five contained gramine. In these plants the alkaloid was not present in the coleoptile, but was detected in the roots and leaves, increasing in concentration in the latter organs from the base to the apex. It was further established that the total gramine content of the plant remains constant from the fourth to the tenth day following germination, but no alkaloid could be detected after one month.

More recently, Marion, *et al.*, in studies on the biogenesis of alkaloids, fed tryptophan labeled with C¹⁴ in the β position to germinating barley seedlings (variety Charlottetown No. 80) and were able to isolate radioactive gramine from the leaves, indicating that tryptophan serves as a precursor to gramine (4). Radioautographs of such barley leaves indicated that the tryptophan was first concentrated near the base of the leaves and then reappeared as gramine in the leaf tips

(5). A further study (6), employing a mixture of tryptophans labeled in the 2 and β positions, verified that the conversion of tryptophan to gramine takes place without cleavage of the indole-alanine linkage.

Because gramine is a derivative of indole, a structure which is of considerable physiological interest, it appeared worth while to obtain more detailed information regarding the ontogenesis of the alkaloid, especially with respect to its reported occurrence in the root as well as its ultimate disappearance from the leaf.

EXPERIMENTAL

Chromatographic Identification of Gramine.—Before proceeding with the ontogenetic phase of the investigation, it was necessary to develop a sensitive process for the separation and identification of the basic nitrogenous compounds occurring in barley seedlings; specifically choline, gramine, and hordenine. The paper chromatographic method appeared well suited for this purpose, but gramine and hordenine proved to be extremely difficult to separate completely, and a large number of the more conventional organic solvent systems were employed without success. Good resolution was finally achieved using an aqueous solution of an electrolyte as suggested by Resplandy (7).

Mixtures of the bases were spotted on 1 × 22.5-in. strips of Whatman No. 1 filter paper and subjected to ascending formation with a 10% aqueous solution of ammonium sulfate for about five hours. A more rapid and equally satisfactory separation was achieved employing the circular formation method

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of Rutter (8). With Whatman No. 1 paper in a 15-cm. Petri dish, time of development was reduced to less than two hours. A particular advantage of this wash liquid was its aqueous character which made it possible to spot comparatively crude extracts of plant material on the chromatogram since many impurities (lipids, chlorophyll, etc.) are insoluble in it and do not move from their original position.

Localization of the bases was carried out by dipping the chromatogram in Munier's reagent (9). Average R_f values obtained by the ascending technique were: gramine 0.64, hordenine 0.78, and choline ran with the solvent front. Corresponding values were obtained by the circular method, but here standard substances were employed for reference purposes.

The Detection of Gramine in Seedlings.—Quantities of Charlottetown No. 80 barley seed¹ were germinated in moist air at room temperature as described by Meyer and Anderson (10). In cases where seedlings in an advanced stage of development (greater than ten days) were desired, the seeds were planted in soil contained in pots and allowed to develop under greenhouse conditions.

Two series of experiments were carried out. In the first, seedlings were grown in total darkness for periods of three, six, and eight days. When harvested, the plants were separated into root, coleoptile, and leaf and dried in a circulating air oven at 45°. Seedlings of the second group were allowed to develop subject to the normal diurnal variations of light and then treated as previously described. In this series, however, the period of development was extended to include the roots and leaves of plants harvested 10, 15, 20, 25, 30, 40, and 50 days after germination. A quantity of ungerminated seed was also tested for gramine, since the presence or absence of the alkaloid in these parts had not been established.

After drying, the various plant parts were ground to a No. 40 powder in a Wiley laboratory mill, weighed, and shaken in stoppered test tubes containing 1 ml. of ethanol per 100 mg. of plant material for one hour. The sample size employed generally ranged from 100 to 500 mg. The tubes were then centrifuged, the extract decanted into a small evaporating dish and reduced to dryness in a stream of warm air. The residue was then redissolved in 1 ml. of ethanol, and a quantity of this extract representing a minimum of 25 mg. of the dried plant material was spotted in small, successive portions on the chromatographic sheet or strip which was then treated as previously described. In this way, 5 μ g. of gramine could be detected with certainty. Expressed in terms of the minimum quantity of extract spotted, this is equivalent to 0.02% of the dry weight of the plant material tested.

RESULTS AND DISCUSSION

Gramine could not be detected in the ungerminated seed or in the coleoptiles of either normal or etiolated seedlings tested three, six, and eight days following germination. In the leaves the alkaloid

was present in both normal and etiolated plants three days after germination, which was the earliest stage of development at which it was possible to effect an efficient separation of the leaf and coleoptile. It continued to be present in the leaves of both series of plants until the experiment was concluded, in the case of the etiolated plants after eight days, in the case of the normal plants, fifty days. Gramine was never detected in the roots of any of the seedlings during the entire course of the experiment.

The discrepancies in these results in comparison to those reported by previous investigators may be explained on the basis of the use of different plant material or a difference in the methods employed. The procedure of Brandt, *et al.* (3), for estimating gramine involves an absorbance measurement at 280 $m\mu$ of a purified alkaloidal extract. In the case of roots tested in this way, the extract would also contain hordenine which absorbs strongly at this wavelength. This may account for the earlier report of gramine in the roots of barley seedlings.

Although there is a possibility that the distribution of gramine as well as its occurrence in the plant is a genetic variable which may differ with different varieties of barley, this hypothesis appears remote in the light of present knowledge. However, this same explanation could account for the existence of gramine in Charlottetown No. 80 barley leaves for at least fifty days following germination, although previous investigators were unable to detect it in month-old plants.

Failure to detect any gramine in the root, coupled with the reports of Marion, *et al.*, regarding synthesis during the initial growth of the leaf, seems to indicate conclusively that the latter organ is the exclusive site of synthesis, as well as storage, of gramine in Charlottetown No. 80 barley. This observation is particularly interesting in view of the numerous observations that the root is the "Hauptbildungsstätte" of many of the alkaloids (11). Although there are exceptions to this rule, such as the shoot synthesis of steroid alkaloids of the solanidine type and anabasin, such examples are not numerous. As Mothes points out, however, it is not too presumptuous to think that the present opinion concerning the locus of alkaloid formation is too limited, being based largely upon sporadic data. In any event, gramine may be added to the comparatively small group of alkaloids which are known to be synthesized and stored in the plant shoot.

REFERENCES

- (1) Paech, K., "Biochemie und Physiologie der Sekundären Pflanzenstoffe," Springer-Verlag, Berlin, Göttingen und Heidelberg, 1950, p. 239.
- (2) Euler, H. v., Hellstrom, H., and Löfgren, N., *Hoppe-Seyler's Z. physiol. Chem.*, **234**, 151 (1935).
- (3) Brandt, K., Euler, H. v., Hellstrom, H., and Löfgren, N., *ibid.*, **235**, 37 (1935).
- (4) Bowden, K., and Marion, L., *Can. J. Chem.*, **29**, 1037 (1951).
- (5) Bowden, K., and Marion, L., *ibid.*, **29**, 1043 (1951).
- (6) Leete, E., and Marion, L., *ibid.*, **31**, 1195 (1952).
- (7) Resplandy, A., *Compt. rend.*, **238**, 2527 (1954).
- (8) Rutter, L., *Nature*, **161**, 435 (1948).
- (9) Block, R. J., Durrum, E. L., and Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press Inc., New York, 1955, p. 245.
- (10) Meyer, B. S., and Anderson, D. B., "Laboratory Plant Physiology," 2nd ed., D. Van Nostrand Company, Inc., New York, 1947, p. 91.
- (11) Mothes, K., "Annual Review of Plant Physiology," Vol. 6, Annual Reviews, Stanford, Calif., 1955, p. 393.

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A Procedure for the Isolation and Determination of Trasentine in Solutions*

By ANTHONY J. TARASZKA and ARNOLD D. MARCUS†

Aqueous solutions of Trasentine® hydrochloride (β -diethylaminoethyl diphenylacetate hydrochloride) may be analyzed for residual concentrations of the intact ester by means of partition chromatography and subsequent nonaqueous titration. Aliquots of the aqueous solutions are introduced onto the silicic acid columns, prepared to contain an internal phase buffered to pH 4.8, as slurries with carbon tetrachloride and silicic acid. The Trasentine base is then selectively eluted with chloroform and the concentration of base determined by titration with standard perchloric acid in glacial acetic acid using α -naphtholbenzein indicator. The results obtained by this method are in good agreement with those obtained by other methods. In addition, the procedure described has a number of advantages for use in conjunction with kinetic studies.

PRIOR TO UNDERTAKING a study of the kinetics of hydrolysis of Trasentine hydrochloride (β -diethylaminoethyl diphenylacetate hydrochloride) it was necessary to choose or develop an analytical procedure which would permit accurate and precise determinations of residual quantities of the intact drug. Although at least three ordinarily acceptable methods were already available, each of these had one or more inherent disadvantages which rendered it unsuitable for our needs.

The gravimetric method (1), based upon formation of the picrate, was entirely too time-consuming. Direct spectrophotometric determination (2) could not be employed because of the similarity in absorption spectra between Trasentine and diphenylacetic acid, one of the major degradation products. The procedure of Goddu, *et al*, for esters (3) was considered, but the nature of the reagents required seemed to introduce the possibility of degradation other than that desired. It appeared, therefore, most plausible to develop a new method.

The present communication indicates the feasibility of employing partition chromatographic separation of the undegraded Trasentine base and subsequent titration of this base with perchloric acid in glacial acetic acid in the presence of a suitable indicator.

DISCUSSION OF THE EXPERIMENTAL APPROACH

Nonaqueous determination of Trasentine as the free base requires that the ester be separated completely from any β -diethylaminoethanol which might result from hydrolysis. This separation is vital inasmuch as both compounds respond as bases in the nonaqueous solvents suitable for elution of Trasentine. Although it would be desirable to effect a similar separation of Trasentine from diphenylacetic acid, this preference is a matter of elegance rather than necessity. The acid does not, as will be shown later, interfere with the titrations.

Because β -diethylaminoethanol is a much stronger base than Trasentine and much more soluble in water, it was assumed that silicic acid columns buffered at pH 6.0 would permit a simple separation of the two amines. In light of the pKa of diphenylacetic acid (*ca.* 4) it also seemed possible to retain the acid on the column while eluting the undegraded ester with chloroform. The flowing chromatogram shown in Fig. 1, which was obtained in the presence of β -diethylaminoethanol, apparently confirmed the suitability of such a procedure. When, however, this method was applied, the precision and accuracy were so poor as to necessitate some change.

The poor results seemed best attributable to either or both of two reasons. Either the Trasentine was being held on the column because of the adsorptive properties of silicic acid, or the ester was undergoing degradation during the course of separation. The first of these alternatives was regarded as unlikely in view of a lack of any significant tailing in the chromatogram. Because it is known (1) that the rate of hydrolysis of Trasentine increases markedly with hydroxyl ion concentration, the second alternative was pursued. Since Trasentine exhibits greatest stability between pH 4-5, the columns were changed to provide an internal phase buffered to pH 4.8. This simple change permitted an excellent order of precision and accuracy without any noticeable alteration in the shape of the chromatogram.

EXPERIMENTAL

Materials.—Trasentine hydrochloride; silicic acid, Mallinckrodt, Chromatography Grade; 0.5 M pH 4.8 acetate buffer; chloroform, Baker analyzed reagent; carbon tetrachloride, N. F.; α -naphthol-

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The authors wish to thank Mr. Jack Cooper of Ciba Pharmaceutical Products, Inc., for his generosity in supplying us with the Trasentine hydrochloride and for making available to us his data relative to analytical procedures and general stability. They also wish to express their appreciation to Mr. Thomas Medwick of the University of Wisconsin, School of Pharmacy, for his valuable suggestion concerning the choice of indicators.

benzen, 0.1% in glacial acetic acid; 0.01 *N* perchloric acid in glacial acetic acid.

Equipment.—Chromatographic columns, 2 (i.d.) \times 40 cm. provided with suitable delivery tubes and indented at the base so as to hold glass wool; close fitting glass plungers for packing the columns; 5- and 10-ml. burets.

Procedure.—Pack the column according to the procedure of Higuchi and Patel (4). Use 20 grams of silicic acid as the holding phase, 20 ml. of 0.5 *M* pH 4.8 acetate buffer as the internal phase, and carbon tetrachloride as the external phase.

Pipet an exact volume (not to exceed 5.00 ml.) of an aqueous solution of Trasentine, adjusted to pH 4.8 and containing between 0.03 and 0.05 Gm. of the drug in each ml. onto a quantity of dry silicic acid numerically equal in grams to the volume of solution used. Mix thoroughly with a small spatula, add 10 ml. of carbon tetrachloride and form a slurry by thorough mixing. Transfer the slurry to the column using quantitative technique and pack it tightly onto the top of the column. When removing the plunger from the column, use care to avoid carrying along any adhering slurry. Rinse the container (preferably a 30-ml. beaker) with an additional 10 ml. of carbon tetrachloride and add the rinsings to the column. When all but the last traces of supernatant liquid have entered the column, force any of the slurry, which may have adhered to the sides of the column, downward onto the main portion. At this point, add a slurry prepared from 1 Gm. silicic acid, 1 ml. buffer, and 5 ml. of carbon tetrachloride to the column and pack down tightly.

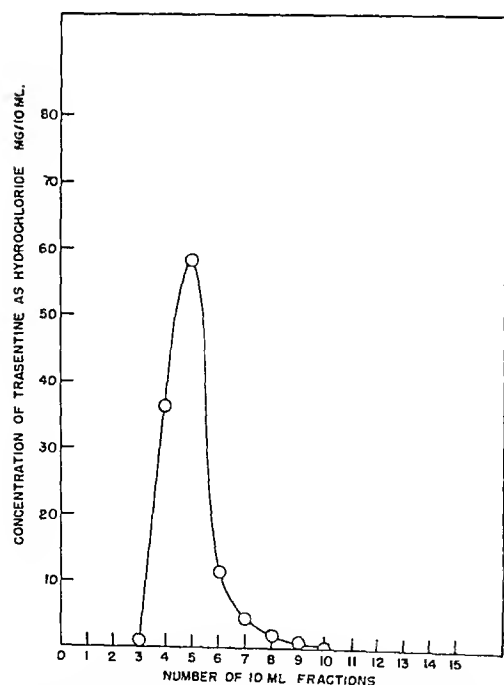


Fig. 1.—Partition chromatogram showing the selective elution of Trasentine from a buffered silicic acid column. In addition to 120 mg. of Trasentine, the sample also contained an equivalent amount of 2-diethylaminoethanol.

When all of the supernatant liquid has again entered the body of the column, pipet exactly 5 ml. of chloroform into the column and begin collecting the eluate in a 100-ml. volumetric flask. Allow all but a bare trace of the chloroform to drain into the column, add a second 5-ml. portion of chloroform and allow it, too, to drain into the column. Chloroform may then be added in larger amounts but the flow rate should not exceed 2 ml. in three minutes.

After 100 ml. of eluate have been collected in the volumetric flask, take 10-ml. aliquots for determination of the Trasentine content. To each 10-ml. aliquot add 5 drops of 0.1% α -naphtholbenzen in glacial acetic acid and titrate with 0.01 *N* perchloric acid in glacial acetic acid to the proper visual end point. Perform a blank to compensate for the basicity of the indicator.

Each ml. of 0.01 *N* perchloric acid used is equal to 3.479 mg. Trasentine hydrochloride.

RESULTS

As shown in Table I, the analytical procedure described above permits quantitative recovery of Trasentine from aqueous solutions.

TABLE I.—RECOVERY OF TRASENTINE FROM AQUEOUS SOLUTIONS

Sample No.	Trasentine HCl Added, mg.	Found, mg.	Recovery, %
1	123.9	123.5	99.7
	123.9	124.6	100.5
	123.9	123.3	99.5
2	116.1	116.2	100.0
	116.1	116.9	100.7
	116.1	116.8	100.7
3	118.4	117.2	99.1
	118.4	117.3	99.1
	118.4	117.5	99.2
4	138.7	138.1	99.6
	138.7	137.4	99.1
	138.7	137.4	99.1

It was stated previously that diphenylacetic acid, even if it were eluted along with the Trasentine base, would not interfere with the titrations. The data in Table II testify to the validity of this assumption. These data were obtained by including an amount of diphenylacetic acid equivalent to that which would be present if all of the Trasentine had hydrolyzed. Since the acid is not completely soluble at pH 4.8, the undissolved acid was allowed to remain in suspension. The suspensions were shaken thoroughly prior to each withdrawal of a sample for

TABLE II.—RECOVERY OF TRASENTINE HYDROCHLORIDE IN PRESENCE OF DIPHENYLACETIC ACID

Sample No.	Trasentine HCl Added, mg.	Found, mg.	Recovery, %
1	118.9	118.3	99.5
	118.9	118.8	99.9
	118.9	118.0	99.2
2	138.5	137.2	99.1
	138.5	137.5	99.3
	138.5	137.9	99.5

analysis. Although this may have detracted somewhat from the accuracy of the method, the essential point is, nevertheless, clearly demonstrated.

SUMMARY AND CONCLUSIONS

1. The concentration of Trasentine hydrochloride in aqueous solutions may be determined by selectively eluting the free Trasentine base from partition chromatographic columns prepared from silicic acid, 0.5 *M* pH 4.8 acetate buffer and carbon tetrachloride. Chloroform is used as the eluant. One of the products of hydrolysis of Trasentine, β -diethylaminoethanol remains on

the column. While it is not certain whether the other product, diphenylacetic acid remains behind, this is of little consequence inasmuch as the acid does not interfere with determination of Trasentine base by nonaqueous titration.

2. The procedure described is apparently precise to within 1% and offers advantages over other methods for use in conjunction with kinetic studies.

REFERENCES

- (1) Cooper, J., Personal communication.
- (2) Rehm, C., Personal communication.
- (3) Goddu, R. F., *et al.*, *Anal. Chem.*, 27, 1251(1955).
- (4) Higuchi, T., and Patel, K. P., *THIS JOURNAL*, 41, 171 (1952).

A Study of the Decomposition of Glucose Solutions*

By NORVAL E. WEBB, Jr.,† GLEN J. SPERANDIO, and ALFRED N. MARTIN

Glucose solutions were thermally decomposed under varying conditions. Preliminary findings showed that the formation of 5-hydroxymethylfurfural from glucose was subject to general acid-base catalysis. Results also indicate that the rate of reaction was inversely proportional to the initial concentration of glucose.

THE INSTABILITY OF GLUCOSE SOLUTIONS is one of the major problems encountered in the field of parenteral medication. Although considerable work is reported in the literature concerning this problem, little seems to have been accomplished in prolonging the shelf life of glucose injections.

Investigators have studied the effects of heat on glucose solutions (1-4), since autoclaving is the most practical method for the sterilization of parenteral products. It has been shown that an excessive amount of heat causes the formation of a yellow color, and the theory has been advanced that at least part of this color is due to the polymerization of 5-hydroxymethylfurfural (hereafter referred to as 5-HMF) (3, 5), a decomposition product of glucose. Singh, *et al.* (6), found that the extent of coloration of glucose solutions

paralleled the extent of 5-HMF formation, the least amount of coloration occurring at pH 3.0. The rate of formation of this product has been assumed to be proportional to the decomposition rate of glucose (7), which would lead to the conclusion that the rate of glucose degradation in solutions could be determined, under varying conditions, by measuring the rate of formation of 5-HMF.

Brönsted and Guggenheim (8) found a general acid-base catalysis in the mutarotation of α -glucose and attributed it to the anions and corresponding undissociated acids. It would be reasonable to assume that the decomposition of glucose to 5-HMF would also be subject to general acid-base catalysis.

It was the purpose of this investigation to determine the effect of varying concentrations of glucose in solution upon the rate of decomposition, to study the effect of different buffering systems as a means of establishing general acid-base catalytic effects, and to prepare an Arrhenius plot to determine the energy of activation for the conversion of glucose to 5-HMF.

EXPERIMENTAL

Materials.—Mallinckrodt A. R. anhydrous dextrose was chosen for use in this study. Redistilled water was used in the preparation of all solutions. The 5-HMF used in preparing the standard spectro-

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photometric curve was obtained¹ as a dark brown viscous liquid and was crystallized and purified by vacuum distillation at 114–117° at a pressure of 1 mm. of mercury. All decomposition runs were carried out using 100-ml., Army type, serum bottles of type-I glass.

Apparatus.—All pH determinations were made with a Beckman model H2 glass electrode pH meter. The Beckman DU spectrophotometer with ultra-violet attachment was used for the assay of 5-HMF, using 10-mm. fused silica cells. The glucose solutions were decomposed in a constant temperature oven, which could be controlled within a temperature range of $\pm 0.5^\circ$.

Assay.—The wavelength of maximum absorbance for a solution of 5-HMF was found experimentally to be 284 $m\mu$. A series of dilutions of 5-HMF in 10% glucose solution was accurately prepared, and the absorbances were determined at 284 $m\mu$ with a distilled water blank. The data were found to follow Beer's law over the range of 0.1 to 1.0. Absorbance was plotted against concentration and a line was fitted to the points by the method of least squares. In order to arrive at specific rate constants, the assay values were converted to mcg. of 5-HMF per mole of anhydrous glucose.

Methods.—With the exception of two runs made for the Arrhenius plot, all decomposition runs were conducted at a temperature of 100°. The solution containers were placed in the oven and attached to a sample-removal device which eliminated opening the oven to obtain the samples. Samples were taken initially and every thirty minutes for a period of two hours.

At the end of each decomposition run the samples were assayed for 5-HMF. After converting the absorbances of these samples to mcg. of 5-HMF per mole of glucose, the concentration of 5-HMF was plotted against time on rectangular coordinate graph paper. In a majority of cases the results were not linear; that is, the rate of formation increased with time, indicating what was considered to be autocatalysis. A typical plot is shown in Fig. 1. Specific reaction rates were determined by drawing a tangent to the origin of the curve by a method similar to that described by Lotshaw (9). The specific rate constant was expressed as k_i = (mcg. 5-HMF per ml./moles of glucose initially present per ml.)/hr., where the subscript (*i*) is used to indicate that the rate was determined from the initial part of the curve.

Concentration Effect upon Decomposition Rate.—A series of glucose solutions varying in concentration from 5 to 25% was decomposed under identical conditions and the specific reaction rates were compared. The solutions were maintained at a pH of 5.3 by the use of an acetate buffer system. The results are shown in Table I. It is seen that as the concentration of glucose increased the rate constant decreased. It is of interest to note that Zheltukhin (10) reported no effect on the rate of decomposition by the concentration of glucose with studies conducted at 145° and 167°; however, at 187° an increase was reported in the rate of decomposition with increased glucose concentration.

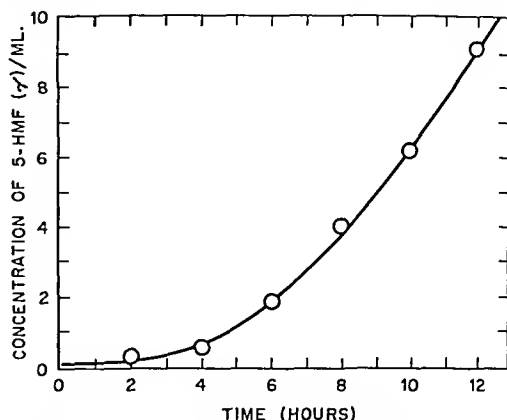


Fig. 1.—The rate of formation of 5-HMF from a 10 per cent anhydrous glucose solution at 100° C.

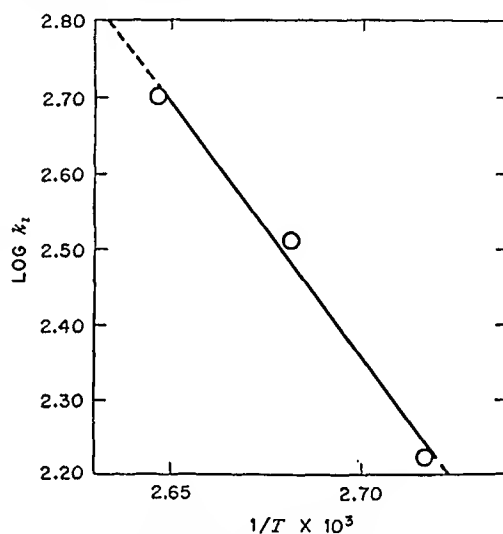


Fig. 2.—The effect of temperature upon the rate of glucose decomposition.

Since the rate of glucose decomposition decreased with the increase in glucose concentration under the conditions used in this study, it would be conceivable that by increasing the concentration of glucose in a buffered solution, and carrying out sterilization at a temperature of about 100°, the stability of glucose solutions for injection could be improved.

General Acid-Base Catalytic Effect.—To determine the acid-base catalytic effect upon the conversion of glucose to 5-HMF, buffered solutions containing 10% glucose were decomposed in the manner

TABLE I.—REACTION RATE CONSTANTS FOR VARYING CONCENTRATIONS OF GLUCOSE

Glucose, %	MCG 5-HMF/mole of Glucose/Hr. ($10^{-2} k_i$)
5	11.0
10	11.0
15	7.0
20	6.5
25	5.4

¹ Purchased from the Bios Laboratories, 17 W. 60th Street, New York 23, N. Y.

previously described. In no case was there any appreciable variation in initial and final pH. Three buffer systems were selected to cover a pH range of from pH 3.0 to pH 6.7. A formic acid-sodium formate system was used at pH 3.0, an acetic acid-sodium acetate system at pH 5.0, and a monobasic potassium phosphate-sodium hydroxide system at pH 6.7. The buffer ion concentration of the buffer system at each pH was varied. The results are shown in Tables II, III, and IV.

TABLE II.—DECOMPOSITION OF 10% ANHYDROUS GLUCOSE IN A FORMATE BUFFER SYSTEM AT A pH OF 3.0

Molarity of Total Formate Including Undissociated Acid	Meg 5-HMF/mole of Glucose/Hr (10^{-4} k)
0.006	2.2
0.012	2.5
0.107	3.2
0.193	5.0

TABLE III.—DECOMPOSITION OF 10% ANHYDROUS GLUCOSE IN AN ACETATE BUFFER SYSTEM AT A pH OF 5.0

Molarity of Total Acetate Including Undissociated Acid	Meg 5-HMF/mole of Glucose/Hr (10^{-4} k)
0.005	1.2
0.010	1.6
0.052	5.6
0.098	9.3
0.213	29.0

TABLE IV.—DECOMPOSITION OF 10% ANHYDROUS GLUCOSE IN A PHOSPHATE BUFFER SYSTEM AT A pH OF 6.7

Molarity of Phosphate Ion	Meg 5-HMF/mole of Glucose/Hr (10^{-4} k)
0.050	3.0
0.100	3.4
0.200	3.8

In each case it can be seen that as the molar concentration of the buffer increased the rate of decomposition was accelerated. It may be concluded from this data that the conversion of glucose to 5-HMF is subject to general acid-base catalysis. These findings indicated that the catalytic effect of anions and undissociated acids of any buffer system that one might wish to employ in the preparation of glucose solutions, must be considered as well as the hydrogen ion concentration of the solution.

Temperature Effect.—For this portion of the study 10% glucose solutions were decomposed at three different temperatures: 95, 100, and $105 \pm 0.5^\circ$. The solutions for decomposition were prepared using a sufficient quantity of hydrochloric acid to give a normality of 0.001. Under these conditions the pH of the solutions remained unchanged throughout the duration of the runs. The results are shown in Fig. 2. The line best fitting the points was determined visually and the slope of the line

was calculated to be -6.7×10^3 . From the classical Arrhenius equation and the value obtained for the slope in Fig. 2, the activation energy was calculated to be roughly 31,000 calories. Although only three runs were made at 5-degree intervals the activation energy agreed well with the average value of 32,800 calories reported by Saeman (11).

It is not possible at this stage of the investigation to employ the decomposition data at elevated temperature for predicting the stability of glucose solutions at room temperature. The results obtained in preliminary experiments at temperatures below 100° were erratic and the autocatalysis ruled out the possibility of obtaining a specific reaction rate that would remain constant over extended periods of time.

SUMMARY AND CONCLUSIONS

1. The rate of formation of 5-HMF was used to obtain information concerning the effect of various conditions upon the rate of decomposition of glucose solution exposed to high temperatures.

2. The results at 100° showed an inverse relationship between the rate of decomposition and the concentration of glucose. This would indicate that perhaps greater stability of the solution could be achieved by increasing the glucose concentration.

3. The conversion of glucose to 5-HMF is subject to general acid-base catalysis as indicated by the increase in the rate of decomposition with increasing concentrations of buffer ions when hydrogen ion concentration is held constant. Therefore, the catalytic effect of ions other than hydrogen must also be considered when preparing buffered glucose solutions.

4. The energy of activation for the conversion of glucose to 5-HMF was found to be approximately 31,000 calories.

5. Further studies on autocatalysis and solvent effects in the conversion of glucose to 5-HMF are underway and will be reported at a later time.

REFERENCES

- (1) Völksen, W., *Arch. Pharm.*, **28S**, 392(1952).
- (2) Hornauer, H., *Pharmazie*, **9**, 574(1954).
- (3) Scalliet, B. L., and Gardner, J. H., *J. Am. Chem. Soc.*, **67**, 1934(1945).
- (4) Hudson, T. A., and Tarlowski, L., *Pharm. J.*, **1S8**, 451(1947).
- (5) Joslyn, M. A., *Ind. Eng. Chem.*, **33**, 308(1941).
- (6) Singh, B., Dean, G., and Cantor, S., *J. Am. Chem. Soc.*, **70**, 517(1948).
- (7) Hirayama, H., and Kubota, T., *Japan J. Pharm. Chem.*, **23**, 387(1951), through *Chem. Abstr.*, **46**, 4748(1952).
- (8) Brønsted, J. N., and Guggenheim, E. A., *J. Am. Chem. Soc.*, **49**, 2554(1927).
- (9) Lotshaw, M., *ibid.*, **47**, 793(1925).
- (10) Zheltukhin, D. V., *Zhur. Priklad. Khim.*, **26**, 882(1953).
- (11) Saeman, J., *Ind. Eng. Chem.*, **37**, 43(1945).

Evaluation of Suspensions Using Electrokinetic Measurements*

By G. L. STANKO† and H. G. DeKAY

The need for methods of evaluating and predicting the stability of suspensions has been recognized. The factors of pH and viscosity do not enable the investigator to predict and evaluate a series of suspensions. Electrokinetic factors appear to be of value as these measurements seem to indicate the stability of a suspension.

NINETY per cent of the drugs used at the present time did not exist ten years ago (1). A great majority of these new drugs are synthetic chemicals possessing characteristics that often make it difficult to formulate a stable suspension. There is also a wide choice of surface active agents, emulsifying agents, flavoring agents, suspending agents, and preservatives that increases the compounding problem. Therefore, with the large number of potential formulas to be considered, a need arises for screening measurements to indicate the stability of the product. These measurements would make it possible to determine more rapidly and discard the unstable formulas early in the formulation study.

The methods generally used for the evaluation of a pharmaceutical suspension have been measurement of viscosity (2), pH (3), sedimentation (4), and particle size (5). Other approaches to the problem have included the use of a shaking machine (6) and a sedimentation-kymograph (7). These studies indicate the suspending ability of a substance for a drug, but do not predict whether agglomeration or caking will occur. In nearly all suspensions, some sedimentation will take place, but the important aspect is whether the sediment can be easily and uniformly resuspended. Thus, there seems to be a need for a method of more quantitatively determining the optimum pH, suspending, and wetting agents so that the most compatible formula can be obtained.

A few reports in the literature have contained references to the electrostatic properties of a suspension (8-11). Incompatibilities were thought to be due to the opposite charges of ions and suspending agent. This aspect of pharmaceutical suspensions has not been very intensely investigated, although it has indications of being an

important factor in determining the stability of a suspension.

The investigation of the stability of carbon in soap solutions resulted in the conclusion that zeta potential was a principal factor in producing a stable suspension (12). The effect of different electrolytes on a vanadium pentoxide sol and a chromium hydroxide sol was measured. When the electrokinetic potential fell below a certain point, the sols became unstable (13). The suspendability of various detergents for a manganese dioxide sol was studied. It was concluded that the magnitude of the zeta potential on the particles was the dominant variable in determining whether or not a stable suspension was obtained (14). In these studies of nonpharmaceutical suspensions, the electrokinetic factors have been shown to be of fundamental importance; therefore, it is the purpose of this paper to consider electrokinetic factors as a method of evaluating and predicting the stability of a pharmaceutical suspension.

EXPERIMENTAL

Suspensions of sulfamerazine in 5% concentration were prepared with the following suspending agents: methylcellulose, 4000 c. p. s. and 25 c. p. s.; sodium carboxymethylcellulose medium and high viscosities; and sodium alginate. The suspending agents were used in 1% concentrations and all were preserved with 0.1% methyl parahydroxybenzoate. Each suspension was prepared with a mortar and pestle, then passed through a hand homogenizer. One hundred milliliters of each suspension was placed in a graduated cylinder for sedimentation observations, and the balance divided into four-ounce prescription bottles. These portions were used for the various periodic measurements in order to have an undisturbed sample. All suspensions were stored at room temperature on the laboratory desk.

Each suspension was observed for sedimentation, static and dynamic, pH, viscosity, and electrokinetic properties. The pH was measured with a line operated Beckman meter and a Hoesppler viscosimeter was used to measure the viscosity. The temperature of the suspension was held at $20^{\circ} \pm 0.2^{\circ}$ during the viscosity measurement by means of a circulating water bath.

The two types of sedimentation studies were made in an attempt to determine the amount of settling and whether agglomeration was taking place. For the amount of settling, a 100-ml. graduated cylinder was filled to the mark with the suspension and sealed. These were then observed

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for settling. A dynamic sedimentation test was similar to the method described by Daniels, *et al.* (15). Ten milliliters of thoroughly redispersed suspension was diluted to 350 ml. with distilled water. An aluminum disk was suspended in the dilute suspension and a 5-mg. weight added to the balance. When the pointer returned to zero, the time was noted and another 5-mg. weight added. This was continued until a definite trend was established as the interest was only in the larger particles.

The electrokinetic properties were studied by measuring the zeta potential and critical potential. Zeta potential was calculated from the following formula (16):

$$Z = (4 \times \pi \times \eta \times \mu) / D$$

For the conductance measurement, a Kohlrausch type slide wire was used with an oscillograph to indicate the null point. The rate of movement of the particles was measured in a microelectrophoresis cell (17). Two hundred and twenty-five volts were imposed across the cell and the time of particle travel was measured with an electric timer measuring to the 0.1 second. Ten readings were taken, five in each direction, at the lower 21% level and averaged to obtain the particle rate of travel.

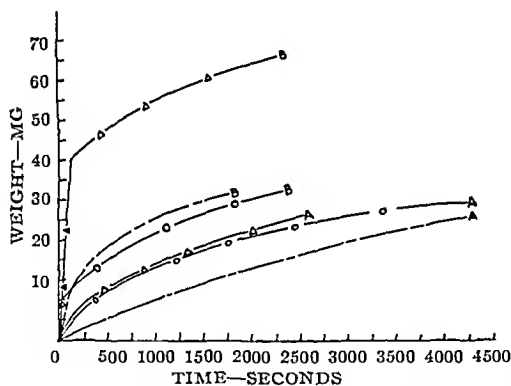


Fig. 1.—Sedimentation rate of 5% sulfamerazine. —△—methyl cellulose 25 c.p.s.; —○—methyl cellulose 4000 c.p.s.; ———CMC—medium viscosity.

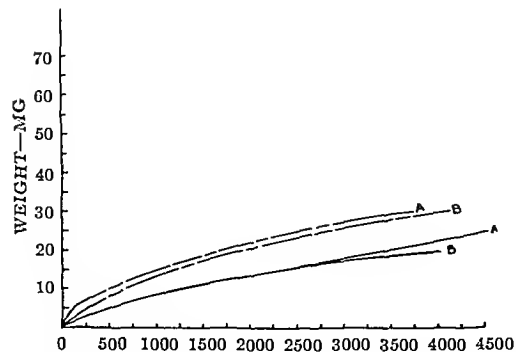


Fig. 2.—Sedimentation rate of 5% sulfamerazine. ———CMC—high viscosity, ———sodium alginate.

RESULTS

The sedimentation observations indicated that some marked changes had taken place. At the end of two months the methyl cellulose 4000 c. p. s. suspension had a cloudy supernatant layer with a caked sediment to the 7-ml. mark. This sediment could be redispersed with difficulty. A translucent zone above the 7-ml. mark was noted in the methyl cellulose 25 c. p. s. suspension. The sediment that rose to the 7-ml. mark could not be redispersed and had to be dug out of the graduate. Although the sodium alginate suspension settled to the 22-ml. mark, this sediment was easily and uniformly redispersed with no apparent caking. A sediment rising to the 10-ml. mark with a translucent supernatant layer was observed with the sodium carboxymethylcellulose medium viscosity suspension. However, this sediment was redispersed with slight shaking and no caking was apparent. No definite layer of powder could be noted with the carboxymethylcellulose high viscosity suspension, but there was a clear zone of supernatant liquid down to the 95-ml. mark. The suspension was easily resuspended with no apparent caking.

The rate of sedimentation test shows graphically what had taken place in the cylindrical graduates. Each suspension was thoroughly shaken to redisperse any sediment before a sample was removed. The methyl cellulose 25 c. p. s. suspension had caked severely and also had a large increase in the rate of sedimentation as shown in Fig. 1. Line *A* is the initial test and line *B* was determined four weeks later. Figure 1 also illustrates the caking that had taken place in the methyl cellulose 4000 c. p. s. suspension. A moderate amount of agglomeration had already taken place. The sedimentation rate of the sodium carboxymethylcellulose medium viscosity suspension also indicated the beginning of agglomeration (Fig. 1), while the high viscosity sodium carboxymethylcellulose suspension showed practically no change in sedimentation rate (Fig. 2). The sodium alginate suspension also remained uniformly dispersed as illustrated in Fig. 2.

These observations show that these suspensions greatly vary. The measurements of pH viscosity are shown in Tables I and II.

TABLE I.—pH OBSERVATIONS

	Initial	Two Weeks	Four Weeks
Methyl Cellulose, 25 c. p. s.	5.9	6.2	5.8
Methyl Cellulose, 4000 c. p. s.	5.3	5.5	5.6
Sodium Carboxymethylcellulose			
Medium viscosity	6.7	6.8	6.5
High viscosity	6.3	6.5	6.3
Sodium Alginate	5.9	6.0	5.7

With the exception of an unexplained decrease in viscosity of the high viscosity sodium carboxymethylcellulose suspension, the measurements of pH and viscosity did not indicate that any changes were taking place in the suspensions. This would seem to indicate that these factors do not predict the stability of a suspension.

TABLE II.—VISCOSITY OBSERVATIONS, C. P. S.

	Initial	One Week	Two Weeks	Four Weeks	Seven Weeks
Methyl Cellulose, 25 c. p. s.	7	7	7	7	7
Methyl Cellulose, 4000 c. p. s.	163	159	165	161	168
Sodium Carboxymethylcellulose					
Medium viscosity	38	39	37	37	36
High viscosity	264	222	222	181	174
Sodium Alginate	40	40	40	39	39

TABLE III.—ZETA POTENTIAL MEASUREMENTS

Suspending Agent	Initial	One Week	Zeta Potential, mv.			Five Weeks	Seven Weeks
			Two Weeks	Three Weeks			
Methyl Cellulose, 25 c. p. s.	39	9	20	19	21	24	
Methyl Cellulose, 4000 c. p. s.	7	16	8	5	7	5	
Sodium Carboxymethylcellulose							
Medium viscosity	133	174	157	104	124	113	
High viscosity	80	142	116	201	139	142	
Sodium Alginate	119	141	123	142	155	126	

The electrokinetic factors were studied by measuring the zeta potential and critical potential. Each measurement was from an aged, undisturbed portion of the original suspension. Table III presents the zeta potentials observed.

Critical potential is considered that zeta potential at which flocculation of the suspension occurs. It is not necessary to reduce the zeta potential to zero to obtain flocculation. As all of the suspensions in this study had negative charges, the addition of a highly positive ion could flocculate the suspension. An ion of this type was found in benzalkonium chloride and was used as the 12.8% solution. The zeta potential was measured in the usual manner, but the benzalkonium chloride was added before the suspension was diluted to the measuring volume. The effect is revealed in Fig. 3. The zeta potential decreased steadily with increasing amounts of benzalkonium chloride until flocculation took place. This point was very apparent as the suspension clumped together in large aggregates and settled rapidly. Flocculation took place at about 29 millivolts, which left a slight charge remaining on the particle. Thus the critical potential of the sodium alginate suspension was considered as 29 millivolts. The other suspensions were not measured for critical potential at this time.

The effect of various percentages of suspending agent on the zeta potential was measured by preparing a series of 5% sulfamerazine suspensions with 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% sodium alginate. The viscosities and zeta potentials were measured in the usual manner. The results are shown in Fig. 4. The peak potential was obtained at 1.5% sodium alginate, but a range of 1.25% to 1.75% would still be near the peak potential. The viscosity in this range varied from 120 to 340 c. p. s., so zeta potential measurements may be an aid in choosing the most favorable concentration of suspending agent.

DISCUSSION

This work was performed to attempt to develop methods of evaluating and predicting the stability of a suspension. Previous reports in the literature mention that the measurements of pH and viscosity do not always indicate whether a particular suspension will or will not be stable. The measurements of pH and viscosity with these suspensions settled and caked without any change in these factors.

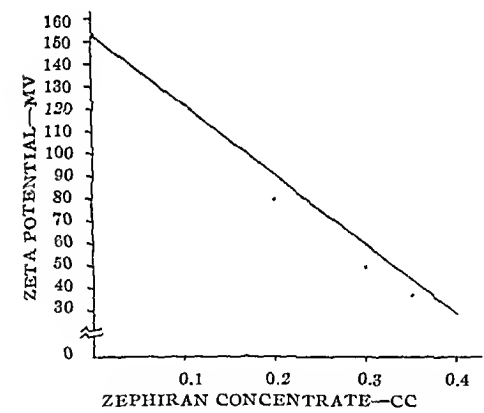


Fig. 3.—Critical potential of 5% sulfamerazine in sodium alginate 1%.

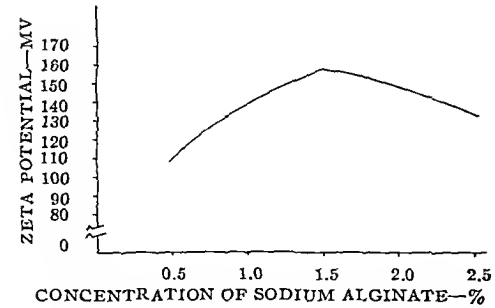


Fig. 4.—Zeta potential of 5% sulfamerazine in various concentrations of sodium alginate.

Electrokinetic factors, such as zeta potential and critical potential, seem to offer a method of evaluating and predicting the stability of a suspension. When the sulfamerazine suspensions were initially measured, the methyl cellulose preparations were much lower in zeta potential than the other suspensions. These suspensions settled and caked. The sodium carboxymethylcellulose medium viscosity suspension showed a decline in zeta potential and also an increase in the rate of sedimentation. By being able to discard immediately potentially unstable suspensions, as the methyl cellulose group, and being able to compare the suspensions during aging, as the sodium carboxymethylcellulose suspensions, the measurement of zeta potential appears to be of value in predicting and evaluating a series of suspensions.

It was shown that a stable suspension can be flocculated when the zeta potential is lowered below a critical level. This factor would be of value after the basic formula is chosen and other ingredients such as preservatives, dyes, and flavoring agents are to be added. These substances can adversely affect the stability and a consideration of the critical potential would offer a method of determining the most compatible combination to use. Also, the concentration of the suspending agent could be chosen by electrokinetic measurements.

The study of these factors proposes a method of screening and evaluating a series of suspensions so

that the most stable preparation can be found. The other factors of pH and viscosity could be adjusted in relation to the electrokinetic factors. Therefore, if each suspension is an individual problem, sufficient measurements are needed to evaluate and compare various formulas.

REFERENCE

- (1) Editor, *American Druggist*, 132, 42(1955)
- (2) Huyck, C. L., *THIS JOURNAL, Pract Pharm Ed*, 11, 170(1950)
- (3) Kerr, W. L., and Zopf, L., *ibid*, 14, 426(1953)
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- (8) Husa, W. J., and Plaxco, J. M., Jr., *THIS JOURNAL, Pract Pharm Ed*, 14, 222(1953)
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- (13) Ghosh, B. N., Chowdhury, R. N. R., and Burman, D. P., *J Indian Chem Soc*, 28, 1(1951)
- (14) Greiner, L., and Vold, R. D., *J Phys & Colloid Chem*, 53, 67(1949)
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- (16) Moyer, L., *J Biol*, 31, 531(1936)
- (17) Abramson, H. A., "Electrokinetic Phenomena and Their Applications of Biology and Medicine," 1st ed., The Chemical Catalog Company, Inc., New York, N. Y., 1934, p. 75

A Simplified Graphic Method for the Preparation of Isotonic Solutions*

By E. ROY HAMMARLUND† and KAJ PEDERSEN-BJERGAARD‡

A new simplified graphic method for the adjustment of isotonic solutions is presented. The use of this method replaces many of the arithmetical calculations which are usually necessary with other methods of adjustment. The amount of NaCl that is required to be added to each solution can be quickly and accurately determined for all concentrations. A revised table of NaCl equivalents for 332 different medicinal substances which are usually used in the preparation of isotonic solutions is presented. The table is more complete than previously used tables and it gives the NaCl equivalents at various concentrations.

THE USE OF ISOTONIC SOLUTIONS has increased markedly in the last few years. Many methods have been proposed for calculating the amount of NaCl, or other substance, that must be added to a hypotonic solution to make it isotonic with blood and tears. Most of these methods make use of some type of NaCl equivalent which is a comparison of the osmotic effect of a solution of a medicinal substance to that of NaCl.

There are numerous tables of NaCl equivalents in the various pharmaceutical texts; but unfortunately, there are some discrepancies in these tables; depending upon the method of calculation for each substance, the freezing point used, and upon whether the values were theoretical or experimentally determined. Since these discrepancies exist in the presently used tables of NaCl equivalents, the purpose of this study is to investigate some of the colligative properties of all drugs whose aqueous solutions are used in collyria, collunaria, injections, or in other contact

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The authors wish to thank Johan Larsen, The Royal Danish School of Pharmacy, and B. C. Smidt, Bispebjerg Hospital, Copenhagen, for making many of the freezing point and vapor pressure measurements. The authors are also grateful to the various manufacturers for supplying many of the preparations used in this study.

This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

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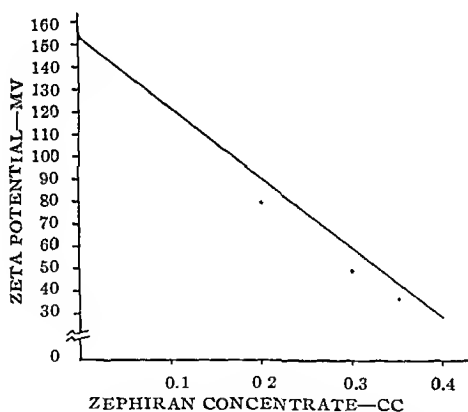


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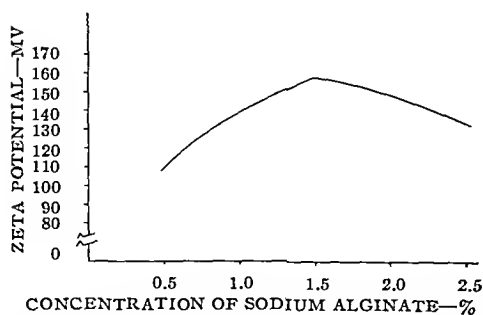


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with tissues; and to present the data both as a complete table of NaCl equivalents at various concentrations, and as a new, simplified graphic method of isotonicity adjustment. The substances tested were of U. S. P. or N. F. grade of purity or better, such as reagent grade, chemically pure, or at whatever grade of purity each specialty is marketed.

EXPERIMENTAL METHODS AND RESULTS

Pedersen-Bjergaard and co-workers (1-3) have previously reported the freezing point depressions of solutions of many medicinal compounds which were calculated from vapor pressure measurements using Baldes' modification (4) of Hill's thermo-electric method (5), or which were determined directly by means of a Beckman thermometer. The freezing point measurements were corrected for the amount of disengaged ice and -0.52° was found to be the freezing point of blood, tears, and of 0.9% NaCl solution (6, 7). The freezing point depression for various concentrations of 332 drugs has been determined using the method corrected for disengaged ice. Since all freezing point measurements of the drugs under study were made in comparison to those of a 0.9% NaCl solution, the data that are presented give solutions that are isotonic with normal saline solution and hence, also isotonic with blood and tears.

The data were initially plotted as freezing point depression *vs.* concentration of drug and the graph included a mirrored NaCl curve (1). A series of concentrations of each substance was selected and from the mirrored NaCl curve was obtained the amount of NaCl that is required to make each of these concentrations isotonic. The amount of NaCl required was plotted against the concentration of each substance in question and a new simplified graph of the type suggested by Baggesgaard-Rasmussen and Jerslev (8) was drawn for each of the medicinal substances. In this method the concentration of the substances to be made isotonic (in Gm./100 cc. or gr./fl. oz.) is plotted as the abscissa of a graph and the grams of NaCl required per 100 cc. of solution or grains NaCl per fluid ounce is plotted as the ordinate. The use of the graphs makes it possible for one to determine rapidly the approximate amount of NaCl for adjustment to isotonicity for all possible concentrations even though a NaCl equivalent may not be available for that particular concentration of drug. The fact that some of the graphs are curved lines shows that the freezing point depression is not always directly proportional to the amount of solute. Therefore, it is sometimes undesirable to use only a single NaCl equivalent for all concentrations as has been presented in previous tables, and a graph is a convenient method for obtaining the actual NaCl equivalency of a substance at all concentrations. The use of this graphic method has been shown to give sufficiently accurate results (9). The graphic methods of use are as follows:

Solutions of a Single Compound.—The concentration of the medicinal compound, expressed in percentage (Gm./100 cc. solution), is sought along the abscissa of the graph.¹ One moves vertically

up the line of the proper concentration until it intersects the medicament curve and then moves either right or left to the ordinate and obtains the value of NaCl to be added in Gm./100 cc. or grains/fluid ounce of total solution. For the adjustment

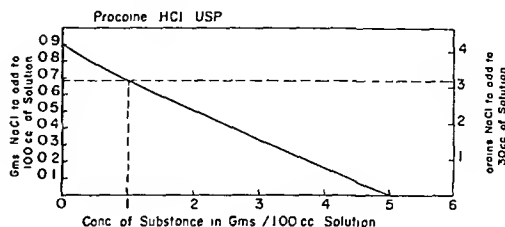


Figure 1

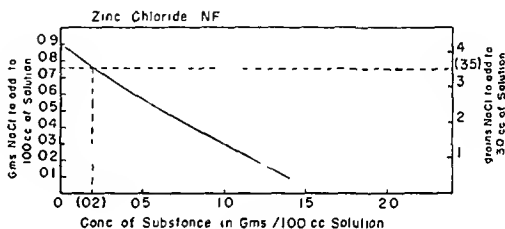


Figure 2

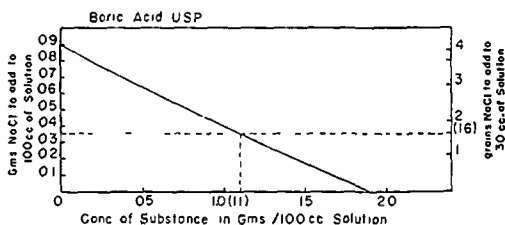


Figure 3

of solutions containing silver ions which are incompatible with NaCl, the graph designates the amount of sodium nitrate to add. Likewise, sodium sulfate should be used for the adjustment of butacaine sulfate solutions.

Example 1

\mathcal{R} Procaine HCl 0.3 Gm.
Distilled H_2O q. s. ad 30.0 cc.
M. Ft. Isotonic Solution

Start from the point on the abscissa corresponding to the concentration of the medicament, 1%; now travel vertically until this concentration cuts the medicament curve; then travel horizontally either left or right depending upon which system of weights is being used. The intersection at the left border is at 0.69 Gm. NaCl/100 cc. of solution. Then,

¹ A booklet which contains the necessary individual graphs to be used for the simplified graphic method of isotonicity adjustment of solutions is being distributed by the authors, free of charge.

$0.3 \times 0.69 \text{ Gm.} = 0.21 \text{ Gm. NaCl}$ for the 30 cc. The intersection at the right border is at 3.2 grains NaCl/fluid ounce which is required to make the prescription isotonic.

This amount of NaCl in Gm. (or grains) which was found to be required can be converted easily to the equivalent number of cc. (or minims) of normal saline solution or any isotonic buffer solution by multiplying the Gm. (or grains) of NaCl needed by 111.1 (or 117.6) and obtaining the volume in cc. (or minims) of isotonic solution to add and then *q. s.* to the final volume with distilled water. This procedure enables one to use previously prepared buffered diluting solutions along with the simplified graphical method and requires only one simple arithmetical operation with a pencil and paper. For example, in the above prescription of Procaine HCl: 0.21 Gm. NaCl is required to make the 30 cc. isotonic and one wishes to use normal saline solution instead of granular NaCl for adjustment; $0.21 \times 111.1 = 23.3 \text{ cc.}$ normal saline solution (or any isotonic solution) to add; then *q. s.* from this volume up to 30 cc. with distilled water and the resulting solution will be isotonic.

Solutions Containing More Than One Compound.—If a solution contains two or more (*n*) medicinal compounds, the amounts of NaCl to be added in order to make each individual compound in the prescribed concentration isotonic are determined in the same manner as described before. In the metric system the sum (*S*) of the required amounts of NaCl (as determined from the individual graphs of (*n*) numbers of ingredients)—minus one less than the total number of ingredients (*n* - 1)—times 0.9 gives the Gm. NaCl which render the prescribed 100 cc. of solution approximately isotonic with blood and tears, i. e., $S - [(n - 1) \times 0.9] = \text{Gm. NaCl required to make 100 cc. solution of } (n) \text{ ingredients isotonic with blood and tears.}$ This amount then may be converted to the corresponding amount of NaCl for the required volume of the prescription if it is different from 100 cc. In the apothecary system, the sum (*S*) of the determined amounts of NaCl in grains/fluid ounce minus $[(n - 1) \times 4.1]$ gives the grains of NaCl which renders one fluid ounce of the solution isotonic. If the result of the subtraction is a negative figure, however, it means the solution is already hypertonic and that no more NaCl should be added.

Example 2

℞ Zinc Chloride	gr. i
Boric Acid	gr. v
Distd. Water, <i>q. s. ad</i>	fl. 5
M. Ft. Isotonic with NaCl	

Zinc Chloride, 1 gr/5, is a 0.2% solution. Boric Acid, 5 gr./5, is a 1.1% solution. 0.2% Zinc Chloride solution requires 3.5 grains NaCl/5, or 0.76 Gm./100 cc. 1.1% Boric Acid solution requires 1.6 grains NaCl/5, or 0.35 Gm./100 cc. Therefore,

3.5 gr/5	0.76 Gm./100 cc.
1.6 gr/5	0.35 Gm./100 cc.
5.1 gr. NaCl	or, 1.11 Gm. NaCl

is the sum of the two ingredients. Substitute in the formula: $5.1 \text{ gr.} - [(2 - 1) \times 4.1 \text{ gr.}] = 5.1 - 4.1 = 1.0 \text{ gr. NaCl required to be added/fl. 5, or, } 1.11 \text{ Gm.} - [(2 - 1) \times 0.9 \text{ Gm.}] = 1.11 - 0.9 = 0.21 \text{ Gm. NaCl required/100 cc. solution. For 30 cc., it is } 0.3 \times 0.21 \text{ Gm.} = 0.06 \text{ Gm. NaCl/30 cc.}$

The required amount of NaCl likewise can be converted to volume of any isotonic diluting solution by multiplying the weight of NaCl in Gm. (or grains) by 111.1 (or 117.6) and obtaining the cc. (or minims) of isotonic solution to add and then diluting to the final volume with distilled water.

$1.0 \text{ gr./fluid ounce} \times 117.6 = 118 \text{ minims normal saline solution to add and then } q. s. \text{ to } 5 \text{ i with water, or, } 0.06 \text{ Gm./30 cc.} \times 111.1 = 6.7 \text{ cc. normal saline solution to add and then } q. s. \text{ to 30 cc. with water.}$

Table of Sodium Chloride Equivalents.—A revised table of NaCl equivalents at 1%, 3%, 5% concentrations, and at the concentration of isotonicity has been prepared on the basis of experimental freezing point determinations and is presented in Tables I and II. Table I gives the NaCl equivalents for those medicinal substances which normally are available to the pharmacist in pure chemical form for use in compounding. Table II gives the NaCl equivalents for those medicinal substances which normally are not available in pure chemical form but occur as prepared solutions and other specialties. One should use the NaCl equivalent which represents the concentration nearest to the desired concentration of medicinal substance used.

TABLE I.—SODIUM CHLORIDE EQUIVALENTS OF DRUGS USUALLY AVAILABLE IN PURE FORM.

Chemical	Concentration of solution			At Isotonicity
	1%	3%	5%	
Acridine N. F.	0.10	0.09
Alcohol U. S. P.	0.65	0.65 (1.39%)
Alcohol, Dehydrated N. F.	0.70	0.70 (1.28%)
Alum (Potassium) N. F.	0.18	0.15	0.15	0.14 (6.35%)
Aminophylline U. S. P.	0.17
Amiodoxyl Benzoate	0.20	0.20	..	0.20 (4.42%)
Ammonium Chloride U. S. P.	1.12	1.12 (0.8%)
Amobarbital Sodium U. S. P.	0.25	0.25	..	0.25 (3.6%)
Amphetamine Phosphate N. F.	0.34	0.27	..	0.26 (3.47%)
Amphetamine Sulfate U. S. P.	0.22	0.21	..	0.21 (4.23%)
Amprotropine Phosphate	0.18	0.16	0.15	..
Amylcaïne HCl	0.22	0.19	..	0.18 (4.98%)
Antimony Potassium Tartrate U. S. P.	0.18	0.13	0.10	..
Antipyrine N. F.	0.17	0.14	0.14	0.13 (6.81%)

TABLE I—(Continued)

Chemical	Concentration of solution			At Isotonicity
	1%	NaCl Equivalents 3%	5%	
Apomorphine HCl U. S. P.	0.14
Arecoline HBr N. F.	0.27	0.24	..	0.23 (3.88%)
Arsenic Trioxide N. F.	0.30
Ascorbic Acid U. S. P.	0.18	0.18	0.18	0.18 (5.04%)
Atropine Sulfate U. S. P.	0.13	0.11	0.11	0.10 (8.85%)
Aurothioglucose N. F.	0.03	0.03	0.03	..
Bacitracin U. S. P.	0.05	0.04	0.04	..
Barbital Sodium N. F.	0.30	0.29	..	0.29 (3.12%)
Benzyl Alcohol N. F.	0.17	0.15
Bismuth Potassium Tartrate N. F.	0.09	0.06	0.05	..
Bismuth Sodium Tartrate	0.13	0.12	0.11	0.10 (8.91%)
Boric Acid U. S. P.	0.50	0.47 (1.9%)
Butacaine Sulfate N. F.	0.20	0.13	0.10	..
Butethamine Formate	0.26	0.21	..	0.20 (4.56%)
Caffeine U. S. P.	0.08
Caffeine and Sodium Benzoate U. S. P.	0.26	0.23	..	0.23 (3.92%)
Caffeine and Sodium Salicylate N. F.	0.21	0.17	0.16	0.16 (5.77%)
Calcium Aminosalicylate U. S. P.	0.27	0.21
Calcium Chloride U. S. P.	0.51	0.53 (1.70%)
Calcium Chloride (6H ₂ O)	0.35	0.36 (2.5%)
Calcium Chloride, Anhydrous	0.68	0.69 (1.3%)
Calcium Gluconate U. S. P.	0.16	0.14
Calcium Lactate N. F.	0.23	0.21	..	0.20 (4.5%)
Calcium Levulinate N. F.	0.27	0.25
Calcium Pantothenate U. S. P.	0.18	0.17	0.17	0.16 (5.5%)
Chiniofon U. S. P.	0.13	0.11
Chloramine-T N. F.	0.23	0.22	..	0.22 (4.1%)
Chlorobutanol (Hydrated) U. S. P.	0.24
Chlortetracycline Sulfate	0.13	0.10
Citric Acid U. S. P.	0.18	0.17	0.16	0.16 (5.52%)
Cocaine HCl U. S. P.	0.16	0.15	0.14	0.14 (6.33%)
Codeine HCl	0.15	0.15
Codeine Phosphate U. S. P.	0.14	0.13	0.13	0.12 (7.29%)
Cupric Sulfate N. F.	0.18	0.15	0.14	0.13 (6.85%)
Cupric Sulfate, Anhydrous	0.27	0.23	..	0.22 (4.09%)
Dextroamphetamine Phosphate	0.25	0.25	..	0.25 (3.62%)
Dextrose U. S. P.	0.16	0.16	0.16	0.16 (5.51%)
Dextrose, Anhydrous	0.18	0.18	..	0.18 (5.05%)
Dibucaine HCl U. S. P.	0.13	0.11	0.08	..
Dihydrostreptomycin Sulfate U. S. P.	0.06	0.05	0.05	0.05 (19.4%)
Emetine HCl U. S. P.	0.10	0.10	0.10	..
Ephedrine HCl N. F.	0.30	0.28	..	0.28 (3.2%)
Ephedrine Sulfate U. S. P.	0.23	0.20	..	0.20 (4.54%)
Epinephrine Bitartrate U. S. P.	0.18	0.16	0.16	0.16 (5.7%)
Ergonovine Maleate U. S. P.	0.16
Ethaverine HCl	0.12
Ethylenediamine	0.44
Ethylhydrocupreine HCl	0.17	0.11	0.09	..
Ethylmorphine HCl U. S. P.	0.16	0.15	0.15	0.15 (6.18%)
Ferric Ammonium Citrate, Green N. F.	0.17	0.15	0.14	..
Ferrous Gluconate U. S. P.	0.15	0.12	0.11	..
Ferrous Lactate	0.21
Fluorescein Sodium U. S. P.	0.31	0.27	..	0.27 (3.34%)
D-Fructose	0.18	0.18	..	0.18 (5.05%)
Galactose	0.18	0.18	..	0.18 (4.92%)
D-Glucuronic Acid	0.20	0.19
L-Glutamic Acid	0.25
Glycerin U. S. P.	0.35	0.35 (2.6%)
Guanidine HCl	0.65	0.61 (1.47%)
Heparin Sodium U. S. P.	0.08	0.07	0.07	..
Hippuran®	0.16	0.15	0.15	0.15 (5.92%)
Holocaine® HCl	0.20
Homatropine HBr U. S. P.	0.17	0.16	0.16	0.16 (5.67%)
Homatropine Methylbromide U. S. P.	0.19	0.15	0.13	..
Hydrastine HCl	0.15	0.12	0.11	..
Hydroxyquinoline Sulfate	0.21	0.14	0.12	..
Hyoscyamine HBr N. F.	0.19	0.16	0.14	..
Hyoscyamine Sulfate N. F.	0.14	0.12	0.11	..
Intracaine HCl	0.23	0.20	..	0.18 (4.97%)
Isoniazid U. S. P.	0.25	0.22	..	0.21 (4.35%)

TABLE I—(Continued)

Chemical	Concentration of solution			
	1%	NaCl Equivalents	5%	At Isotonicity
Lactic Acid U. S. P.	0.41	0.39 (2.3%)
Lactose U. S. P.	0.07	0.08	0.09	0.09 (9.75%)
Magnesium Chloride	0.45	0.45 (2.02%)
Magnesium Sulfate U. S. P.	0.17	0.15	0.15	0.14 (6.3%)
Mannitol N. F.	0.17	0.17	0.18	0.18 (5.07%)
Menadione Sodium Bisulfite U. S. P.	0.20	0.18	0.18	0.18 (5.07%)
Meperidine HCl U. S. P.	0.22	0.20	..	0.19 (4.80%)
Mephensin N. F.	0.19
Merbromin N. F.	0.14	0.11	0.09	..
Mercuric Cyanide	0.15	0.14	0.13	..
Mercury Bichloride U. S. P.	0.13	0.12	0.10	..
Methacholine Chloride U. S. P.	0.32	0.28 (3.21%)
Methadone HCl U. S. P.	0.18	0.14	0.12	0.10 (8.59%)
Methamphetamine HCl U. S. P.	0.37	0.33 (2.75%)
Methenamine U. S. P.	0.23	0.24	..	0.25 (3.68%)
Methionine N. F.	0.28
Methylatropine Bromide	0.14	0.13	0.13	0.13 (7.03%)
Monoethanolamine N. F.	0.53	0.51 (1.76%)
Morphine HCl U. S. P.	0.15	0.14
Morphine Nitrate	0.19	0.15
Morphine Sulfate U. S. P.	0.14	0.11	0.09	..
Narcotine HCl	0.10	0.08	0.08	..
Neomycin Sulfate U. S. P.	0.11	0.09	0.06	..
Nicotinamide U. S. P.	0.26	0.21	..	0.20 (1.49%)
Nicotinic Acid U. S. P.	0.25
"Panthesine"	0.18	0.15
Papaverine HCl U. S. P.	0.10
Potassium Penicillin G U. S. P.	0.18	0.17	0.16	0.16 (5.48%)
Sodium Penicillin G U. S. P.	0.18	0.16	0.16	..
Pentobarbital Sodium U. S. P.	0.25	0.23
Pentylene-tetrazole U. S. P.	0.22	0.19	..	0.18 (4.91%)
Phenindamine Tartrate U. S. P.	0.17	0.12	0.10	..
Phenobarbital Sodium U. S. P.	0.24	0.23	..	0.23 (3.95%)
Phenol U. S. P.	0.35	0.32 (2.8%)
Phenylpropanolamine HCl	0.38	0.35 (2.6%)
Physostigmine Salicylate U. S. P.	0.16
Physostigmine Sulfate	0.13	0.12	0.12	0.12 (7.74%)
Pilocarpine HCl U. S. P.	0.24	0.22	..	0.22 (4.08%)
Pilocarpine Nitrate U. S. P.	0.23	0.20
Polymyxin B Sulfate U. S. P.	0.09	0.06	0.04	..
Potassium Chlorate N. F.	0.49	0.48 (1.88%)
Potassium Chloride U. S. P.	0.76	0.76 (1.19%)
Potassium Iodide U. S. P.	0.34	0.34 (2.59%)
Potassium Nitrate N. F.	0.56	0.56 (1.62%)
Potassium Permanganate U. S. P.	0.39
Potassium Phosphate N. F.	0.46	0.43 (2.08%)
Potassium Phosphate, Monobasic	0.44	0.41 (2.18%)
Potassium Sulfate	0.44	0.43 (2.11%)
Procainamide HCl U. S. P.	0.22	0.19	0.17	..
Procaine HCl U. S. P.	0.21	0.19	0.18	0.18 (5.05%)
Propylene Glycol U. S. P.	0.45	0.45 (2.0%)
Pyridoxine HCl U. S. P.	0.37	0.29
Quinidine Sulfate U. S. P.	0.10
Quinine Bisulfate N. F.	0.09	0.09
Quinine Dihydrochloride N. F.	0.23	0.19	0.18	0.18 (5.07%)
Quinine Hydrochloride U. S. P.	0.14	0.11
Quinine and Urea Hydrochloride N. F.	0.23	0.21	..	0.20 (4.5%)
Racephedrine HCl N. F.	0.31	0.30	..	0.29 (3.07%)
Resorcinol U. S. P.	0.28	0.27	..	0.27 (3.3%)
Scopolamine HBr U. S. P.	0.12	0.12	0.12	0.11 (7.85%)
Scopolamine Methylnitrate	0.16	0.14	0.13	0.13 (6.95%)
Secobarbital Sodium U. S. P.	0.24	0.23	..	0.23 (3.9%)
Silver Nitrate U. S. P.	0.33	0.33 (2.74%)
Mild Silver Protein N. F.	0.17	0.17	0.16	0.16 (5.51%)
Strong Silver Protein N. F.	0.08	0.05	0.04	..
Sodium Acetate, Anhydrous	0.77	0.76 (1.18%)
Sodium Acetate N. F.	0.46
Sodium Aminosalicylate U. S. P.	0.29	0.28	..	0.27 (3.27%)
Sodium Antimonyl Tartrate	0.13	0.12	0.12	0.11 (7.9%)
Sodium Arsenate, Dibasic	0.25	0.24	..	0.24 (3.83%)
Sodium Ascorbate	0.33	0.30	..	0.30 (3.0%)

TABLE I—(Continued)

Chemical	Concentration of solution			At Isotonicity
	1%	NaCl Equivalents	5%	
Sodium Benzoate U. S. P.	0.40	0.40 (2.25%)
Sodium Bicarbonate U. S. P.	0.65	0.65 (1.39%)
Sodium Biphosphate, Anhydrous	0.46	0.43 (2.10%)
Sodium Biphosphate, U.S.P.	0.40	0.37 (2.45%)
Sodium Biphosphate (2H ₂ O)	0.36	0.32 (2.77%)
Sodium Bisulfite U. S. P.	0.61	0.60 (1.5%)
Sodium Borate U. S. P.	0.42	0.35 (2.6%)
Sodium Bromide U. S. P.	0.57
Sodium Cacodylate N. F.	0.32	0.28	..	0.27 (3.3%)
Sodium Carbonate, Anhyd.	0.70	0.68 (1.32%)
Sodium Carbonate, Monohydrated U. S. P.	0.60	0.58 (1.56%)
Sodium Chloride U. S. P.	1.00	1.00	1.00	1.00 (0.9%)
Sodium Citrate U. S. P.	0.31	0.30	..	0.30 (3.02%)
Sodium Hypophosphite N. F.	0.61
Sodium Iodide U. S. P.	0.39	0.38 (2.37%)
Sodium Lactate	0.55	0.52 (1.72%)
Sodium Metabisulfite	0.67	0.65 (1.38%)
Sodium Nitrate	0.68	0.66 (1.36%)
Sodium Nitrite U. S. P.	0.84	0.83 (1.08%)
Sodium Phosphate Exsiccated, N. F.	0.53	0.51 (1.75%)
Sodium Phosphate N. F.	0.29	0.27	..	0.27 (3.33%)
Sodium Phosphate, Dibasic, (2H ₂ O)	0.42	0.40 (2.23%)
Sodium Phosphate, Dibasic, (12H ₂ O)	0.22	0.21	..	0.20 (4.45%)
Sodium Propionate N. F.	0.61	0.61 (1.47%)
Sodium Riboflavin Phosphate	0.08	0.08
Sodium Salicylate U. S. P.	0.36	0.36 (2.53%)
Sodium Sulfate N. F.	0.26	0.23	..	0.23 (3.95%)
Sodium Sulfate, Anhydrous	0.58	0.56 (1.61%)
Exsiccated Sodium Sulfite N. F.	0.65
Sodium Thiosulfate N. F.	0.31	0.30 (2.98%)
Sorbitol (1/2 H ₂ O)	0.16	0.16	0.16	0.16 (5.48%)
Stibamine Glucoside	0.14	0.11
Streptomycin Sulfate U. S. P.	0.07	0.06	0.06	..
Strychnine HCl	0.18
Strychnine Nitrate N. F.	0.12
Sucrose U. S. P.	0.08	0.09	0.09	0.10 (9.25%)
Sulfacetamide Sodium U. S. P.	0.23	0.23	..	0.23 (3.85%)
Sulfadiazine Sodium U. S. P.	0.24	0.22	..	0.21 (4.24%)
Sulfamerazine Sodium U. S. P.	0.23	0.21	..	0.20 (4.53%)
Sulfapyridine Sodium	0.23	0.21	..	0.20 (4.55%)
Sulfathiazole Sodium N. F.	0.22	0.20	..	0.19 (4.82%)
Tannic Acid N. F.	0.03	0.03	0.03	..
Tartaric Acid N. F.	0.25	0.23	..	0.23 (3.9%)
Tetracaine HCl U. S. P.	0.18	0.15	0.12	..
Tetraethylammonium Bromide	0.33	0.28	..	0.28 (3.17%)
Tetraethylammonium Chloride	0.34	0.33 (2.67%)
Theophylline U. S. P.	0.10
Thiamine HCl U. S. P.	0.25	0.22	..	0.21 (4.24%)
Tubocurarine Chloride U. S. P.	0.13	0.10	0.09	..
Urea U. S. P.	0.59	0.55 (1.63%)
Urethan U. S. P.	0.31	0.31 (2.93%)
Viomycin Sulfate	0.08	0.07	0.07	..
Zinc Chloride N. F.	0.61
Zinc Phenolsulfonate N. F.	0.18	0.17	0.17	..
Zinc Sulfanilate	0.21	0.19	0.18	..
Zinc Sulfate U. S. P.	0.15	0.13	0.12	0.12 (7.65%)
Zinc Sulfate, Dried	0.23	0.21	..	0.20 (4.52%)

TABLE II.—SODIUM CHLORIDE EQUIVALENTS OF DRUGS NOT USUALLY AVAILABLE IN PURE FORM

Chemical	Concentration of Solution			At Isotonicity
	1%	NaCl Equivalents	5%	
Adrenaline HCl	0.27	0.22	..	0.21 (4.24%)
9-Aminoacridine HCl	0.17
Amydracaine HCl	0.24	0.18	0.16	0.16 (5.74%)
Amydracaine Nitrate	0.19	0.17	0.16	0.16 (5.68%)
Antazoline HCl U. S. P.	0.23

TABLE II—(Continued)

Chemical	Concentration of Solution			At Isotonicity
	1% NaCl	3% Equivalents	5%	
Antazoline Phosphate	0.20	0.17	0.15	
Aranthol®	0.23	0.23		0.23 (3.96%)
Atropine Methyl Nitrate	0.18	0.15	0.14	0.14 (6.52%)
Benoxinate HCl	0.18	0.14		
Benzalkonium Chloride U. S. P.	0.16	0.14	0.13	..
Benzethonium Chloride U. S. P.	0.05	0.02	0.02	
Benzpyrinium Bromide	0.20	0.18	0.17	
Bromodiphephedramine HCl	0.17	0.10	0.07	
Butethamine HCl N. F.	0.25			
Carbachol U. S. P.	0.36			0.32 (2.82%)
Cetyltrimethyl Ammonium Bromide	0.09	0.09	0.08	
Chlorcyclizine HCl U. S. P.	0.17	0.09	0.07	
Chloresium®	0.10	0.06	0.05	
Chlorpheniramine Maleate U. S. P.	0.17	0.12	0.09	
Chlorpromazine HCl	0.10	0.05	0.03	
Corneceine®	0.18	0.15	0.13	0.12 (7.30%)
Cyclomethycaine Sulfate	0.13	0.10	0.09	
Cyclopentamine HCl	0.36			0.34 (2.68%)
Cyclopentolate HCl	0.20	0.18	0.17	..
Decamethonium Bromide	0.25	0.20	0.18	0.18 (5.0%)
Dibutoline Sulfate	0.16	0.15	0.14	
Dichlorophenarsine HCl	0.55			0.55 (1.64%)
Dicyclomine HCl	0.18	0.17		
Diethylcarbamazine Citrate	0.14	0.14	0.14	0.14 (6.29%)
Dihydrocodeinone Enolacetate HCl	0.14	0.13	0.12	0.12 (7.76%)
Dihydrohydroxycodone	0.14	0.13	0.13	0.12 (7.4%)
Dihydromorphinone HCl U. S. P.	0.22	0.17	0.15	0.14 (6.39%)
Diphenhydramine HCl U. S. P.	0.28	0.20	0.17	
Diphenmethanil Methylsulfate	0.15			
Dipyrene®	0.19	0.19		0.19 (4.65%)
Edrophonium Chloride	0.31	0.27		0.27 (3.36%)
Ephedrine Lactate	0.26	0.24		0.24 (3.72%)
Epinephrine HCl	0.29	0.26		0.26 (3.47%)
Erythromycin Glucoheptonate	0.07	0.07	0.07	
Ethylmeprobamate HCl	0.32	0.28		0.27 (3.32%)
Evans Blue U. S. P.	0.06	0.05	0.05	
Ferric Cacodylate N. F.	0.09	..		
Gallamine Triethiodide	0.08	0.08	0.08	
Glucosulfone Sodium	0.16	0.13	0.13	
Glyphylline	0.12	0.10	0.09	0.08 (10.87%)
Hexamethonium Bromide	0.22	0.19		0.18 (4.99%)
Hexamethonium Chloride	0.27	0.27		0.27 (3.3%)
Hexobarbital Sodium N. F.	0.26	0.24		0.23 (3.88%)
Hexylcaine HCl	0.26	0.22		
Histalog®	0.51			0.47 (1.91%)
Histamine Phosphate U. S. P.	0.25	0.23		0.22 (4.1%)
Histidine Monohydrochloride N. F.	0.29	0.26		
4-Homosulfanilamide HCl	0.28	0.25		
Hydralazine HCl	0.37			
Hydroxyamphetamine HBr U. S. P.	0.26	0.25		0.24 (3.71%)
Iodophthalin Sodium U. S. P.	0.17	0.12	0.11	0.09 (9.58%)
Iodopyracet U. S. P.	0.11	0.11	0.10	0.10 (9.21%)
Iodopyracet Diethylamine	0.12	0.11	0.11	0.10 (8.73%)
Lidocaine HCl N. F.	0.22	0.21		0.20 (4.42%)
Lobeline HCl	0.16			
Menadione Diphosphate	0.25	0.22		
Mephenteramine Sulfate U. S. P.	0.22	0.20		0.19 (4.74%)
Mercaptopurine Sodium U. S. P.	0.18	0.18	0.18	
Mercurophylline U. S. P.	0.13	0.10	0.09	
Mersalyl U. S. P.	0.12	0.11	0.10	0.10 (9.06%)
Methacholine Bromide	0.28	0.24		0.24 (3.77%)
Methantheline Bromide U. S. P.	0.15	0.09	0.07	..
Methapyrilene HCl U. S. P.	0.19	0.18	0.17	
Methoxamine HCl U. S. P.	0.26	0.24		0.24 (3.82%)
Naphazoline HCl N. F.	0.27	0.24		0.22 (3.99%)
Naphuride Sodium®	0.10	0.10	0.10	
Ncoarsphenamine	0.40			0.39 (2.32%)
Neostigmine Bromide U. S. P.	0.22	0.19		
Neostigmine Methylsulfate U. S. P.	0.20	0.18	0.17	0.17 (5.22%)
Nikethamide U. S. P.	0.18	0.16	0.15	0.15 (5.94%)
Novaldin®	0.25	0.20	0.18	0.18 (5.0%)

TABLE II—(Continued)

Chemical	Concentration of solution			At Isotonicity
	1%	NaCl Equivalents	5%	
Oxophenarsine HCl U. S. P.	0.24	0.24	..	0.24 (3.67%)
Oxytetracycline HCl U. S. P.	0.13	0.08
Pentolinium Tartrate	0.17	0.15	0.15	..
Phenarson Sulfoxylate	0.33	0.29	..	0.29 (3.07%)
Pheniramine Maleate N. F.	0.16	0.14	0.13	..
Phenylephrine HCl U. S. P.	0.32	0.30	..	0.30 (3.0%)
Phenylephrine Tartrate	0.19	0.16	0.16	0.15 (5.9%)
Phenylpropylmethylamine	0.38	0.33 (2.7%)
Piperocaine HCl U. S. P.	0.21	0.19	0.17	..
Piridocaine HCl	0.24
Plaquenil® Phosphate	0.18	0.15	0.13	..
Pramoxine HCl	0.18	0.15	0.10	..
Probarbital Calcium	0.25
Probarbital Sodium N. F.	0.32	0.29	..	0.29 (3.1%)
Promethazine HCl N. F.	0.18	0.10	0.07	..
Propoxycaine HCl	0.19	0.16	0.15	..
Pyrimamine Maleate U. S. P.	0.18	0.11	0.09	..
Quinacrine HCl U. S. P.	0.18
Quinacrine Methanesulfonate	0.11	0.10	0.10	..
Quinidine Gluconate N. F.	0.12	0.10
Sodium Folate	0.12	0.10
Sodium Ricinoleate	0.10	0.09	0.09	..
Sodium Sulfadimidine	0.21	0.19	0.18	..
Stibophen U. S. P.	0.18	0.16	0.15	..
Streptomycin Calcium Chloride Complex	0.20	0.19	0.18	0.18 (5.0%)
Streptomycin HCl	0.17	0.16	0.16	..
Succinylcholine Chloride U. S. P.	0.20	0.20	..	0.20 (4.48%)
Sulfisoxazole Diethanolamine	0.18	0.15
Sympocaine® HCl	0.18	0.15	0.14	..
Synkamin® HCl	0.32
Synthenate® Tartrate	0.19	0.17	0.15	..
Tetracycline HCl U. S. P.	0.14	0.10
Tetrahydrozoline HCl	0.28	0.23
Thiopental Sodium U. S. P.	0.27	0.26	..	0.26 (3.5%)
Tolazoline HCl U. S. P.	0.34	0.30	..	0.30 (3.05%)
Transentine® HCl	0.22	0.15	0.12	..
Tribromoethanol U. S. P.	0.05
Trimethaphan Camphorsulfonate	0.10	0.09	0.09	..
Triplennamine HCl U. S. P.	0.30	0.20
Tropacocaine HCl	0.25	0.20	..	0.18 (4.92%)
Trypsarsamide U. S. P.	0.20	0.20	..	0.19 (4.62%)
Tuaminoheptane Sulfate N. F.	0.27	0.27	..	0.26 (3.4%)
Unan®	0.12
Vinbarbital Sodium	0.26	0.25	..	0.25 (3.55%)

SUMMARY

A simplified graphic method for the adjustment of isotonic aqueous solutions has been devised.

A revised table of NaCl equivalents at various concentrations for 332 different medicinal substances which are used in the preparation of isotonic solutions is presented.

The percentage concentration (w/v) of the solute, which when used alone will produce an aqueous solution that is isotonic with blood and tears, is also given in the table.

REFERENCES

- (1) Lund, C. G., Peülicke Nielsen, E., and Pedersen-Bjergaard, K., "The Preparation of Solutions Isoosmotic with Blood, Tears, and Tissue," The Danish Pharmacopoeia Commission, Binar Munksgaard, Copenhagen, 1947.
- (2) Lund, C. G., Pedersen-Bjergaard, K., and Becker Rasmussen, E., *Acta Pharm. Intern.*, 1, 3(1950).
- (3) Lund, C. G., and Pedersen-Bjergaard, K., *Science*, 109, 149(1949).
- (4) Baldes, E. J., *J. Sci. Instr.*, 11, 223(1934).
- (5) Hill, A. V., *Proc. Roy. Soc. Lond.*, A 127, 9(1930).
- (6) Krogh, A., Lund, C. G., and Pedersen-Bjergaard, K., *Acta Physiol. Skand.*, 1, 88(1945).
- (7) Grove-Rasmussen, K. V., Pedersen-Bjergaard, K., and Smidt, B. C., *Acta Pharm. Intern.*, 2, 343(1953).
- (8) Baggesgaard-Rasmussen, H. and Jerslev, B., *J. Pharm. Pharmacol.*, 7, 957(1955).
- (9) Hammarlund, E. R., and Pedersen-Bjergaard, K., *Dansk Tidsskr. Farm., Suppl.* II, 107(1956).

The Kinetics of Rectal Absorption I.*

Preliminary Investigations into the Absorption Rate Process

By SIDNEY RIEGELMAN and WILFRED J. CROWELL†

The details of a radiological procedure are presented by which it is possible to conduct continuous external detection of the rate of rectal absorption of radio-tagged compounds from the specially designed suppository vehicles inserted in the rectal passage of a female rat. An analysis of the rate data is presented and a mathematical expression is derived which corrects the activity detected by the counter for the effect of the diffusion of the drug within the vehicle and for the accumulation of the absorbed drug in the scanned volume segment of the animal. Experimental results on three radio iodide-131 tagged compounds are presented and appear to confirm the resulting pseudo-first order rate equation.

THE MEDICINAL USE of suppositories dates back to the time of Hippocrates in which a vehicle consisting of soap and honey was used for administering cathartic compounds. Active interest in this form of medication developed during the nineteenth century, and since that time, considerable research has appeared both on individual bases and the drugs used therein. From studies based on individual drugs, clinicians have concluded that suppositories usually result in a low rate of drug release and in an erratic rate of absorption when compared to oral or other means of drug administration. Academic researchers, in contrast, have maintained that the rectal route possesses certain theoretical physiological advantages which should enhance the rate of drug absorption over rate obtained by the oral route and that it should be utilized to a much greater extent in modern therapeutics (1-3).

Considerable attention has been given in the literature to research which has endeavored to modify the properties and physical characteristics of cocoa butter, glycerinated gelatin, polyoxyethylene polymers, and numerous other bases. A review of these studies has been published recently by Gross and Becker (4).

Both *in vivo* and *in vitro* procedures have been used to characterize the advantages or disadvantages of proposed formulations and to compare the rectal route to other modes of drug administration. *In vitro* studies have been used to evaluate the rate of drug release by microbiological agar cup methods (5, 6) or by periodically determining the amount of drug dissolving in an aqueous medium from an immersed suppository (7, 8).

Such studies confirm the postulate that water miscible formulations release medicaments at a faster rate than oleaginous bases. However, the rate differences obtained in these studies are of little value in predicting rates of rectal absorption since the solution rate process *in vivo* markedly differs from that in *in vitro* studies.

In vivo studies may be divided into two groups: one group comprising what may be called single point absorption studies; the other group, multi-point absorption or true rate studies. In general, single point studies are based on certain pharmacological end points, such as: loss of the righting reflex due to sedation (9), onset of curarization (10), or a single blood (11) or urine (12, 13) drug level. In contrast to single point absorption determinations, a number of multi-point absorption studies have been conducted to evaluate at different time intervals the level of a drug in the blood (14-19), urine (20), or in selected tissues (15, 16, 18); or to measure peripheral rise in temperature following the rectal administration of methyl nicotinate (21, 22).

Although these single point and multi-point *in vivo* absorption studies present much meaningful data, they provide an indirect approach to the absorption problem. They are based on the assumption that the rate of rectal absorption bears a constant, direct relationship to the amount of drug concentrated by some organ, or the blood level required to elicit a pharmacological response. Such studies provide information suitable only for gross comparisons; not one of them includes a direct procedure for continuous detection of the rate of absorption of the drug occurring at its site of insertion.

This study presents details of a method which was developed to provide direct and continuous detection of the rate of absorption of compounds tagged with a radioactive gamma emitter, I-131, from the rectum of an intact rat. The method

* Received April 22, 1957, from the School of Pharmacy, University of California Medical Center, San Francisco, Calif. This paper is based in part on a thesis submitted to the Graduate School of the University of California by W. J. Crowell in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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results in a high degree of reproducibility of results. Analysis of the rate process is presented and the evidence appears to confirm a pseudo-first order system.

EXPERIMENTAL

Reagents.—Iodoform, Mallinckrodt, recrystallized twice from 95% ethanol, *m p* 118–120°; 2,4,6-triiodophenol, Eastman, recrystallized from 95% ethanol, *m p* 155.5–158°; Tween 20, Atlas Powder Co., and Antaro[®] A400,¹ General Dyestuffs Corp., were individually heated to 60° under reduced pressure (0.3 mm Hg) to remove moisture and any low boiling fractions. Each compound was characterized by estimating the molecular weight from a measurement of the freezing point of a benzene solution of the surfactant. Polyethylene glycol 6000 pharmaceutical grade, Carbon & Carbide Co., was crystallized once from a solvent system containing 20% anhydrous ether and 80% chloroform. The degree of purity of both the crystallized and uncrystallized samples was determined by freezing point lowering measurements made with a calibrated thermistor-resistance meter (23). The molecular weight of the polyethylene glycol calculated from these values indicated a compound with a molecular weight considerably less than 6000. Polyethylene glycol 600, Carbon and Carbide Co., pharmaceutical grade; methylcellulose 6000 c.p.s. grade, Dow Chemical Co.; sodium carboxymethylcellulose high viscosity grade, Hercules Powder Co.

Radiological Equipment and Procedures.—All of the work done with radioactive isotopes was confined to the Radioactivity Center of the University of California Medical Center. Detection of the gamma radiation of sodium iodide-131 was accomplished by use of a scintillation counter composed of a shielded RCA 5819 photomultiplier which sensed the scintillations excited by the radiations elicited by the radio-tagged dose in a thallium activated sodium iodide crystal. This signal was coupled to a preamplifier and fed through a Tracer Laboratory Autoscaler, model SC-IB (Fig. 1). Due to the complex gamma decay scheme of iodide-131, it is not possible to give an absolute efficiency rating to the equipment, but it was in the order of magnitude of 20%.

The actual labeling of iodoform (IF) and triiodophenol (TIP) with iodine-131 was carried out in a well ventilated fume hood, the sides and front of which were lined with lead bricks to a height of 22 inches. The lead floor of the hood was covered with stainless steel trays lined with strips of parafilm so that an accidental spill might be conveniently confined and the working area readily decontaminated. Centrifugation procedures were conducted in the reaction area and the supernatant liquids and washings collected in wide-mouth, glass-stoppered containers which were stored in a lead vault for disposal purposes. Surgical gloves were worn and the manipulations were carried out using a special pair of tongs (a test tube holder was clamped to the jaws of a pair of surgical tongs to facilitate

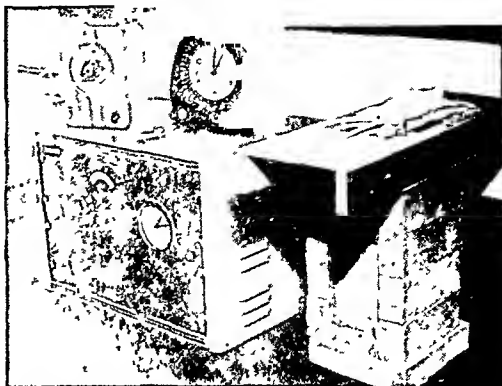


Fig. 1.—A photograph illustrating the position of the anesthetized rat, placed over the collimator-scintillation counter so as to obtain maximal, continuous external detection of a gamma-emitting dose. The scintillation counter, enclosed by lead brick shielding to minimize background counting rate, is electrically connected to the autoscaler and to the timer at the left of the rat.

the handling of small containers of radioactive material).

The scale of reaction for the preparation of IF and TIP was calculated by dividing the total activity of I-131 by the specific activity required for the rectal absorption studies.

Scale of reaction—total activity available/required specific activity— $(4 \times 1000 \text{ mcc.})/200 \text{ mcc./mg.} = 20 \text{ mg.}$

Synthesis of Iodoform (IF).—Labeled IF was prepared according to the method of Vogel (24) by transferring a calculated volume of I-131 of total activity of 3 to 4 mc. to a 12-ml. centrifuge tube. A 0.25-ml. quantity of an aqueous preparation containing 25 mg. of sodium iodide ($1.67 \times 10^{-4} M$) and 3.95 mg. of acetone ($6.8 \times 10^{-5} M$) was added to the radioactive solution, followed by the addition of 0.40 ml. of 5% sodium hypochlorite solution.

After one hour the precipitated IF was collected by centrifugation and washed successively with a small volume of water and a 5% solution of potassium iodide. The IF was then dissolved in approximately 0.2 ml. of hot 50% aqueous ethanol. This solution was cooled in an ice bath to effect crystallization and the IF again collected by centrifugation, *m. p.* 118–120°; radioactive iodide impurities, 1–2%. The compound was dissolved in polyethylene glycol 600 for safe storage.

Synthesis of Triiodophenol (TIP).—The synthesis of labeled TIP was carried out according to a modification of the method of Marsh (25) by transferring a calculated volume of NaI-131 of total activity of 3 to 4 mc. to a 12-ml. centrifuge tube containing 2 ml. of glacial acetic acid, 19 mg. sodium iodide ($12 \times 10^{-4} M$) and 4.0 mg. of phenol ($4.2 \times 10^{-5} M$). To the clear solution, 0.05 ml. of 30% hydrogen peroxide was added with a minimum of stirring. After a twenty-four hour interval, 8 ml. of water was added to the reaction mixture. The precipitated TIP was collected by centrifugation and washed successively with 2-ml. volumes of 5% potassium iodide aqueous solution.

Following the washing and centrifuging procedures, the TIP was dissolved in approximately 1

¹ This compound is a polyethylene glycol ether of nonyl phenol acid is now sold under the trade name of Igepal[®] CO-630.

ml. of hot aqueous ethanol solution, allowed to crystallize in an ice bath, and was collected by centrifugation; m. p. 155.5–158°; radioactive iodide impurities, a mere trace. The compound was dissolved in polyethylene glycol 600 for safe storage.

Method for Determining Radiochemical Purity of Labeled Compounds.—The radiochemical purity of IF and TIP was determined by ascending paper partition chromatography, followed by analysis of the chromatogram using radioautographic techniques. Pure benzene was used as the solvent. Approximately 5 microcuries of labeled substance, dissolved in equal parts of ethanol and PEG 600, was applied to the base of the Whatman No. 1 filter paper and chromatographed for three hours. The solvent benzene at the base of the sealed jar was monitored for activity and found to possess negligible amounts. The paper strip was carefully dried and placed in contact with an x-ray film for one hour. The darkened areas on the developed film were used as a criterion for cutting the corresponding chromatogram into two nearly equal parts, one part containing the base line and the other part containing the labeled compound. The individual parts were folded several times, stapled to assume a fixed geometry, and monitored by means of a scintillation counter. Impurities in the sample, such as radioactive iodide, were then determined by expressing counts per second for the baseline portion as a per cent of the pooled count recorded for the entire paper strip.

Method for Determining the Activity of Labeled Compounds.—Since both IF and TIP are slightly volatile compounds, no attempt was made to dry them to a constant weight nor to standardize them by doing an absolute beta count. The activities, however, were determined relative to a solution of NaI-131, standardized by absolute beta count, which was transferred in volumetric increments to several 12-ml. centrifuge tubes containing milligram quantities of inert sodium iodide. The increments, covering a range of 8 to 500 microcuries of I-131, were assayed for gamma radiation by placing them individually in a lead container on the collimator of a standardized (RaD-RaE) scintillation counter. By plotting counts per second *versus* known activity on log-log coordinate paper, a graph was obtained for the conversion of IF and TIP gamma emissions into I-131 microcuries equivalents.

Pharmacological Procedures.—Female Sprague-Dawley rats, weighing 180 to 205 Gm., were anesthetized with sodium pentobarbital in a 20 mg. per ml. solution by interperitoneal injection in concentrations of 4.3 to 4.7 mg. per 100 Gm. body weight. In the majority of cases surgical anesthesia was complete in seven to fifteen minutes and persisted for about ninety minutes. At that time the experimental procedure was terminated and the animals were sacrificed by administering a lethal dose of undiluted pentobarbital solution.

Prior to the experiment the fecal content in the rectal canal was reduced by fasting the rat for thirty-six to forty-eight hours.² At the end of the fasting period, the rat was anesthetized and its rectum prepared by inserting a moistened plug to a depth of 2.5 cm. using a wetted solid-glass rod appli-

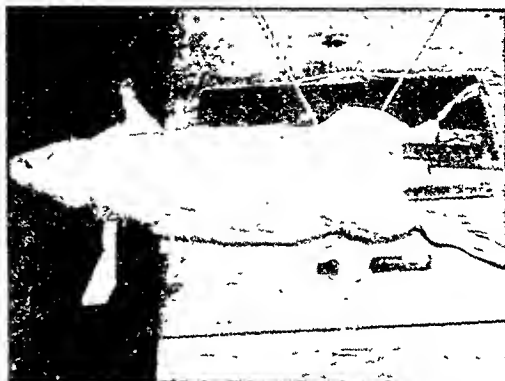


Fig. 2.—A photograph showing the proximal and distal rectal plugs used to confine a radiotagged dose to a definite length of the rat's rectal canal. The scanning field of the counter is depicted by the crossed sticks placed in the one-inch cylindrical lead collimator.

cator. This plug, referred to as the proximal rectal plug, was constructed by inserting a glass bead (3.5 mm. in diameter) into a small portion of rubber tubing. The viscous suppository vehicles were retained in the rectum by the distal plug which was constructed by pulling a trimmed rubber policeman over a hollow glass tube (2 mm. in diameter). This plug was inserted into the anal canal to a depth of one centimeter and held in place by adhesive tape. The combination of plugs left a cavity of 1.5 cm. into which the dose of 0.2 ml. was inserted.

After preparing the rectum, the anesthetized rat was mounted in the supine position with its legs stretched and taped to a plexiglass board (12 in. \times 5 in. \times 0.2 in.). The board and rat were then placed over a one-inch collimator in the desired geometrical position as determined in preliminary studies to result in maximal counting from the rectal area (Fig. 2). A 0.2 ml. dose was drawn up into a 1-ml. tuberculin syringe fitted with a No. 17 gauge needle. The needle was slipped into the glass tube portion of the distal plug, and forced past the seal of the policeman. In order to minimize the possibility of perforating the wall of the colon, the length of the needle was modified. After injecting the dose, the needle was withdrawn.

Counting Procedure.—A few seconds after introducing the radioactive preparation into the rectal canal, the autoscaler was activated and the counting recorded at one minute intervals for the first twenty minutes, and at five minute intervals for the remainder of the experiment. The scaler was automatically activated by a cam arrangement at one- and five-minute intervals by means of an electronic device driven by a small synchronous motor. Counts per second *versus* time were then plotted on semilog paper for the first ten minutes and extrapolated to zero time. The extrapolated value obtained was taken as 100% of the total dose administered and was used to express all other counts on a per cent basis.

Formulas for Suppository Vehicles.—In the sodium iodide studies, 5 microcuries of NaI-131 was added for each 0.2 ml. of vehicle used. The basic

² The animals were given only water during this period.

formula for the methylcellulose (MC) vehicle and the carboxymethylcellulose (CMC) vehicle is as follows

Methylcellulose, 4000 c p s or	
Carboxymethylcellulose high	
viscosity type	1 5 Gm
Sodium iodide (carrier)	0 167 Gm
Sodium bisulfite	0 050 Gm
Sodium chloride	q s to make isotonic
Phosphate buffer (0 05 M pH	
7 4)	q s to make 100 0 cc

Special MC and CMC vehicles were formulated to contain specified amounts of the nonionic surfactants, Tween 20 (TW 20) and Antarox A400 (A400). These were added to the above vehicles in the required amounts and corrected for, in the tonicity adjustment, and in the quantity of gum added to bring the preparation to constant viscosity. The CMC vehicle was used with the A400 system since a coacervate formed when this surfactant was used in the MC vehicle.

The iodoform was incorporated in a special isotonic liquid polyethylene glycol vehicle (LPEG) at a level of radioactivity of 5 microcuries per 0 2 ml of vehicle used, polyethylene glycol 6000—59 0 Gm, polyethylene glycol 600—5 0 Gm, distilled water—q s to make 100 0 cc.

Triiodophenol was added to a buffered methylcellulose vehicle H in such a way that the sodium phenolate ion was formed (NaTIP). Once again

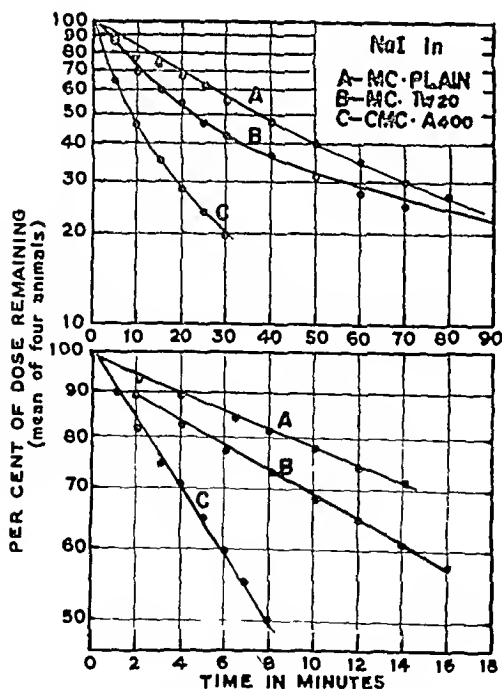


Fig 3—Rectal absorption curves for sodium iodide from three suppository vehicles MC-plain, MC-Tw20-1%, CMC-A400 1%. The upper curves represent the total period of observation, the lower curves represent the initial 18 minute portion of the observed data.

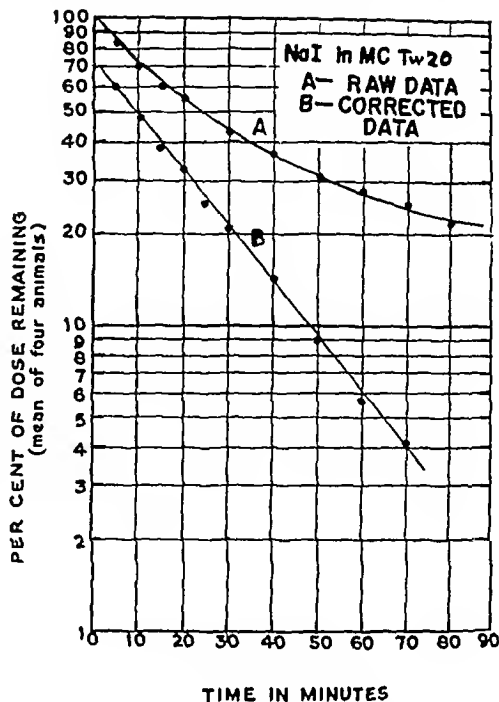


Fig 4—Corrected and uncorrected rectal absorption curves for sodium iodide in MC-Tw20-1%. Curve A represents the uncorrected data while curve B represents the data corrected using Eq (8) or (9).

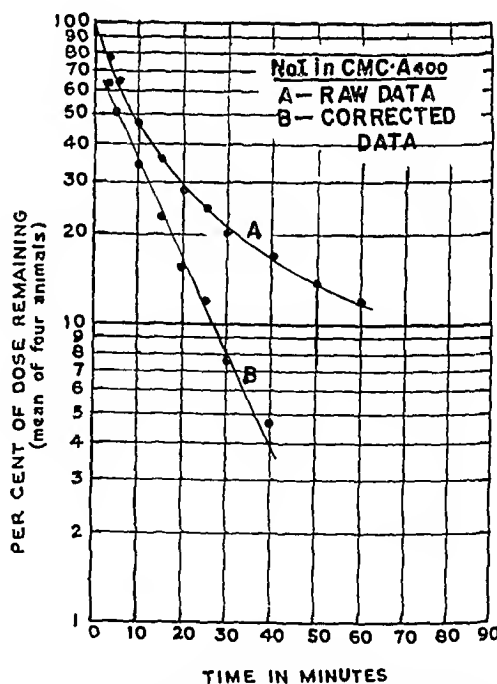


Fig 5—Corrected and uncorrected rectal absorption curves of sodium iodide in vehicle CMC-A400-1%. Curve A represents the uncorrected data while curve B represents the data corrected using Eq (8) or (9).

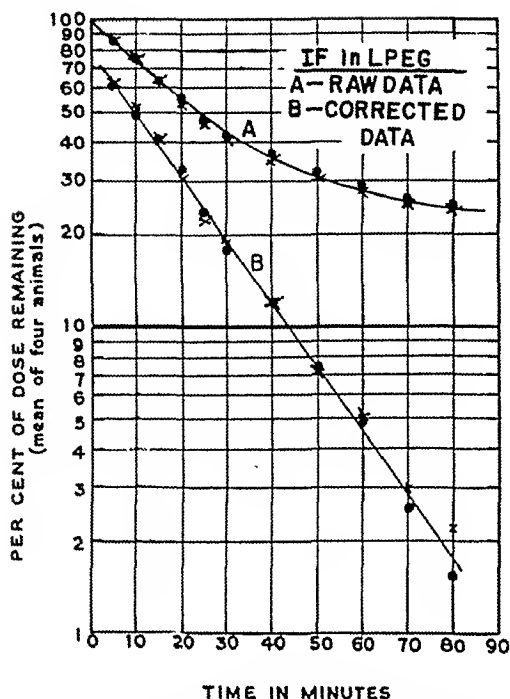


Fig. 6.—Corrected and uncorrected rectal absorption curves for iodoform in the LPEG vehicle at two different concentrations: 64 mcg./0.2 ml. (crosses), and 32 mcg./0.2 ml. (circles). Curve A represents the uncorrected data, while curve B represents the data corrected using Eq. (8) or (9).

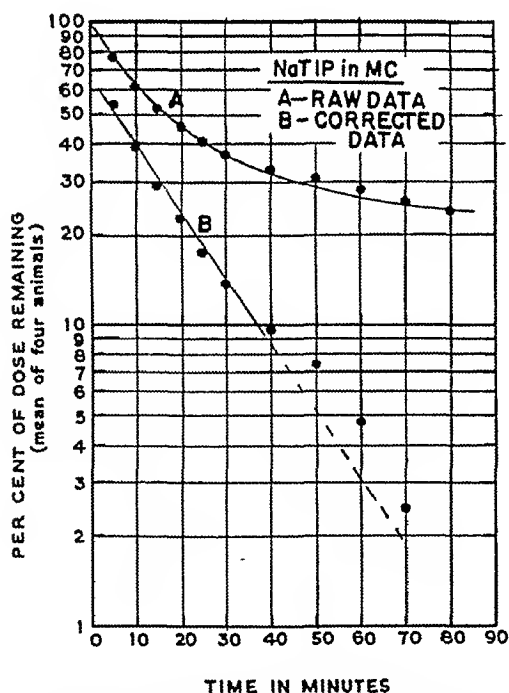


Fig. 7.—Corrected and uncorrected rectal absorption curves for the sodium salt of 2,4,6-triiodophenol in MC-plain vehicle. Curve A—raw data; curve B—corrected data.

5 microcuries per 0.2 ml. of vehicle was used: methylcellulose 4000 c. p. s.—1.5 Gm.; NaCl—*q.s.* to make isotonic; pyrophosphate buffer (0.025 M, pH 8.6)—*q.s.* to make 100.0 cc.

Physical Characterization of the Suppository Vehicles.—Before incorporating the labeled compounds into the suppository vehicles, the isotonicity, pH, surface tension, and viscosity were evaluated for each preparation. The vehicles were made isotonic to a 0.9% solution of sodium chloride. A thermistor resistance meter was used to check the freezing point lowering of the adjusted solution. It was impossible to evaluate directly the osmotic characteristics of the undiluted vehicle by the above freezing point method without dilution, since the vehicle separated free solid glycol on cooling. In this case, the vehicle was diluted twelvefold and data obtained from the diluted solution were used for the adjustment of the tonicity.

The viscosities of the prepared vehicles were adjusted to 750 ± 50 centipoises at 25° using a Cannon Fenske viscometer, size 500. Preparations possessing a viscosity higher than 800 were made less viscous by the addition of small volumes of isotonic, buffered solution; those giving readings lower than the accepted value were made more viscous by the addition of small amounts of appropriate thickening agent. The pH was determined with a Beckman pH meter, model G, and readjusted when necessary. All buffers were designed to possess high buffer capacity at the specified pH. Surface tension measurements were made at room temperature with a Cenco-DuNouy interfacial tensiometer.

RESULTS

A typical set of results is shown for NaI Figs. 3, 4, and 5; for IF in Fig. 6; and for NaTIP in Fig. 7.

DISCUSSION

In the development of a reproducible technique several factors were found to be of critical importance, these included: (a) the degree of anesthesia, which had to be deep enough to eliminate body and peristaltic movements; (b) geometric placement of the dose and its retention within this rectal segment by confining plugs; (c) the viscosity of the vehicles which prevented leakage beyond the plugs; and (d) the type of collimation of the scintillation counter. A scintillation counter, unless restricted by a suitable collimator, will detect any radiation emitted within an 180-degree arc. Such a counter would respond not only to the dose remaining in the rectum, but also would detect most of the absorbed dose. In order to restrict the view of the counter in so far as possible to the rectal volume segment containing the radioactive dose, several preliminary collimation studies were carried out using both cone and cylindrical type collimators attached to a scintillation counter. The isosteric patterns of each collimator were determined by detecting a known radioactive source, positioned at various vertical and horizontal coordinates relative to the crystal of the counter. It was observed that a one-inch cylindrical collimator (Fig. 2) restricted the volume segment of the animal to approximately the first four

centimeters of a rat's rectal canal; in addition, it adequately excluded from the scanned area the liver, kidneys, and other vital organs of the animal.

Studies were also conducted using a probe scintillation counter. It was believed that the small size of the sensing crystal of the probe counter inserted directly into the rectal canal would make it highly efficient in detecting the radioactive dose immediately adjacent to it, but relatively less sensitive to the radioactive molecules accumulating in surrounding tissues. Although the probe counter responded in accordance with its proposed characteristics, difficulty was experienced in placing and maintaining it in the rectum. It also scanned to a certain degree the whole animal, and for these reasons it was not used in this study.

It was found that the data followed an absorption pattern which indicated certain characteristics typical of a first-order rate process. For example, in studies using NaI in MC vehicle, it was observed that by doubling or halving the concentration of the drug, the observed time for 50% absorption was not appreciably changed. Beyond the first half-life (usually around 60% absorption), the NaI data deviate from linearity (Fig. 3, curve A). While this linearity through one half-life may be tacitly taken as evidence of a first-order process, the deviation should be logically explainable.

Two possible explanations perhaps account for the deviation of the NaI data from first-order: following its absorption, a portion of the NaI returns to the volume segment of the animal scanned by the counter; and in the rectal segment, physical changes occur in the vehicle which retard the rate of NaI release for absorption.

In this investigation, the experimental design is such that the volume segment scanned by the counter remains constant in respect to the total volume of the animal. The amount of absorbed drug which returns to this volume segment may also be considered a constant relative to the total amount of absorbed drug; in effect, it is a measure of the ratio of the volume segment to the total volume of the animal. These interrelationships are based on the fact that none of the drug is excreted or exhaled during the experimental procedure. It might be conjectured that the scanned area might abnormally concentrate the drug; however, the drug distribution studies contradict this contention.

The effect of the returning drug, therefore, is not a variable but a constant related to the volume fraction of the animal scanned. While this effect is constantly present, it only corrects the curvature about 5%. In order to rectify the curved portion of the plotted data to the straight line, a total correction of 20 to 5% is required.

A second explanation of the curvature could be based on an assumption that diffusion of the drug, within the vehicle, to the absorption site becomes the rate limiting step which, in turn, would modify the rate of absorption. When vehicles are formulated with surfactants, NaI is absorbed at a significantly faster rate than in the plain vehicles. The faster absorption apparent in these systems results in greater curvature. These results, shown in Fig. 3 (curves B and C), are in accordance with the assumption that the limiting factor is the diffusion of the drug to the absorption site. In the slow absorbing systems the drug can diffuse to the absorp-

tion site at a rate which maintains first-order absorption for a considerable period. With the surfactant systems, one might postulate that the increase in rate of absorption is due to slight damage to the membrane barrier or that the surfactant "cleans" the membrane barrier and thus increases the number of absorption sites. In either case the surfactant causes an increased rate of absorption. Diffusion to the absorption site becomes the rate dependent variable sooner in these systems. Further discussion of the effect of anionic compounds and surfactant suppository systems will be the subject of a separate report (26).

Fortunately the analysis of the rate process for experimental conditions used in these studies may be handled mathematically by analogy to the general solution for radial diffusion (27). The following derivation is for such a rate equation, and includes the correction for the diffusion within the vehicle and for the accumulation of the radioactive substance in the volume segment scanned by the counter. It is possible that if the animals were not under such deep anesthesia, peristaltic processes would eliminate the diffusion variable. The corrected data, therefore, may be more representative of rectal absorption under normal physiological conditions than are the uncorrected data.

DERIVATION OF MODIFIED ABSORPTION EQUATION

N_0 = The total dose administered (detected by the scintillation counter). N = The dose detected by the counter at time, t . f = The volume fraction of the animal being detected by the counter modified by local variations in drug distribution.

N_r = The amount of the dose remaining in the confined rectal section at time, t .

N_u = The amount of the dose remaining unabsorbed at end of the experiment.

N_b = The amount of the total dose absorbed at time t , and distributed throughout the body.

N_f = The dose detected by the counter at the end of the experiment.

Since none of the dose is excreted during the experiment,

$$N_0 = N_r + N_b \quad (1)$$

At any time during the experiment,

$$N = N_r + f(N_b) \quad (2)$$

or substituting for N according to Eq. (1), we have

$$N = N_r + f(N_0 - N_r) \quad (3)$$

Solving for N_r , we have:

$$N_r = [N - f(N_0)] / (1 - f) \quad (4)$$

In our work the experimental design is such that a diffusion gradient is set up within the rectal section. Since we are, in effect, isolating a section of the rectal cavity and following absorption from its membranous surface, we can refer to our section as simulating a finite cylinder with sealed end faces, diffusion out of the cylinder occurring in a radial direction only.³ We are not concerned with

³ It is obvious that the rectum does not meet the exact dimensions of a cylinder; since the cross-section probably resembles an ellipse in shape. It can be shown, however, that such considerations merely change the absolute values of the constants in the final equation. See Jost, W., "Diffusion in Solids, Liquids, Gases," Academic Press Inc., N. Y., N. Y., 1952, p. 35.

the concentration of the drug at any point along the radius of the cylinder; instead, we are following the average drug concentration, defined above as N_r with an initial concentration N_0 and a final concentration relating to the total unabsorbed drug, N_u . Radial diffusion according to these conditions has been solved by Dünwald and Wagner (27) using Fourier analysis; the solution is:

$$(N_r - N_u)/(N_0 - N_u) = \sum_{i=1}^{\infty} 4/X_i^2 \exp [-(X_i^2)^2 D t / r_0^2]. \quad (5)$$

Here r_0 represents the radius of the rectal cylindrical section, D is the diffusion coefficient, and the X_i are the roots of the equation $J_0(X) = 0$, where $J_0(X)$ is the Bessel-function of the zero-th order and second kind ($X_1 = 2.405$, 8.654 , 11.792 , —). From an estimation of r_0 and D it may be shown that for $t > 3$ min., the first term of the series is a good approximation for the complete equation, therefore:

$$(N_r - N_u)/(N_0 - N_u) = 4/(2.405)^2 \cdot \exp [-(2.405)^2 D / (r_0^2) \cdot t] \quad (6)$$

or

$$(\log N_r - N_u)/(N_0 - N_u) = -kt + (\log C) \quad (6a)$$

$$\text{Where } k = r_0^2 / (2.303)(2.405)^2 D; \quad C = 0.7^4$$

Since our counter detects N instead of N_r we must substitute for the latter according to Eq. (4).

$$\log \frac{[N - f(N_0)/(1-f)] - N_u}{N_0 - N_u} = -kt \quad (7)$$

Rearranging and collecting terms we arrive at:

$$\log \frac{N - N_u - f(N_0 - N_u)}{(1-f)(N_0 - N_u)} = -kt \quad (8)$$

We have spoken of a correction term relating to the accumulation of the drug in the volume segment scanned by the counter. It should be noted that Eq. 8 contains no variable function relating to this correction; it may be shown, however, that the constant, f , is the term covering this accumulation. This phenomenon has been demonstrated repeatedly at various time intervals by sacrificing the animals and removing the rectum. The removed rectal portion is then placed on the counter in a geometrical position duplicating as near as possible that in the intact animal. Such a procedure results directly in an evaluation of N_r and by rearranging Eq. 1 to

$$N_0 - N_r = N_b.$$

we can directly evaluate the amount of the dose absorbed, N_b . By placing the animal (minus the rectal portion) back in its proper geometry, the counter detects only the dose in the volume segment. The latter is N of Eq. 2 where N_r becomes zero, and we then have

$$N/N_b = f.$$

Under our experimental conditions, the constant, f , thus determined is well approximated by the value

⁴If we consider the rectum as possessing a different geometric shape, the absolute values of K and C change. For example, for the first term of the series: $C = 0.7$ for a cylinder, and $C = 0.9$ for a slab. The value of C only modifies our intercept. Recognizing that our equation is a good approximation only at $t > 3$ min we can neglect the second term on the right hand side of Eq. (6a).

— 0.05. It does not vary to a large extent even when following different drugs or markedly different types of formulations.

Taking a typical set of experimental results, the value scanned by the counter at the end of the experiments N_f where: $N_f = N$ (90 min.) = 0.25; $N_u = 0.20$; and $f = 0.05$. Substituting these values into Eq. (8), we have

$$\log [N - 0.05(1-0.20) - 0.20] / (1 - 0.20)(1 - 0.05) = -kt$$

or

$$\log N - 0.24/0.76 = -kt.$$

This correction rectifies the raw data to a straight line function which holds over three to four drug half-lives.

It should be noted that a further simplification can be made in Eq. 8 in light of the detailed results given for the typical case listed above. The correction function is nicely approximated within the accuracy of our animal data by using the last determined experimental count detected by the counter on the intact animal, viz. $-N_f$. In the above example, Eq. 8 would result in a correction function of

$$\log N - N_f/N_0 - N_f = -kt$$

or

$$\log [N - 0.25]/0.75 = -kt.$$

Any procedure which reduces the research workers exposure to gamma radiation is a valuable one. The use of Eq. 9 in place of Eq. 8 is well justified, since it eliminates the necessity of additional close contact with the radioactive source during the excising of the rectum.

The derivation does not include any mathematical consideration of the rate process for drug transfer across the rectal membrane. In general, we believe that the absorption rate process is much faster than the diffusion process, i.e., $k_{abs.} \gg k_{diff}$.

Although the apparent rate constant, k , for Eq. 9 is complex in nature, including: diffusion, absorption, and differences in formulation, it is extremely useful in expressing our data and making comparative analysis of different suppository systems.

Confirmation of the Modified Absorption Equation.—Curve *A* in Figs. 4 and 5 represents a plot of the uncorrected data; Curve *B* of these figures is a plot of the corrected data as obtained using Eq. 8, or 9. Discrepancies between the absorption half-lives for the raw and corrected data can be attributed to several causes. Since a curvature is present to a slight degree even in the early data, the 50% absorption time from the raw data will tend towards a longer $t_{1/2}$ than the corrected data. The derivations of Eq. 9 implicitly assumes that the diffusion coefficient did not change with relative changes in drug concentration, that the ideal diffusion process was undisturbed by peristaltic processes, or that mixing due to temperature gradients did not take place.

Although the data presented thus far are only for NaI studies, it was found also that IF and TIP studies could be analyzed in the same manner. In each case, the data conformed to criteria for a pseudo-first-order absorption rate process. For example, when IF was used in the LPEG vehicle in concentrations of 33 mcg./0.2 ml. and 64 mcg./0.2 ml., the data expressed in Fig. 6 is obtained. When

the concentration of IF is doubled in LPEG, no apparent difference is observed in the absorption rate. Data obtained for TIP in vehicle, as is shown in Fig. 7, also meet the criteria for the pseudo-first-order absorption process.

The deviations about the straight line drawn for the corrected data (curve B) are more apparent than real; the lower per cent values amplify the apparent error.

A definite advantage of the derivation is that it arrives at a pseudo-first order equation as a natural consequence of accounting for the diffusion phenomenon. Although it fails mathematically to consider the rate process across the rectal membrane, there is good indication that absorption rate process of the drug is much faster than the diffusion process in the vehicle, that is, $k_{abs} \gg k_{diff}$. In general, the derivation corrects the rectal absorption data through two to four half-lives.

The form of the derivation of the absorption equation and its subsequent confirmation appears to justify the use of first-order kinetic analysis of the experimental results. Yet this procedure is not free from some uncontrolled variables. The exact correction factor which may be applied to rectify the data appears to be somewhat dependent on: the time at which the experiment is arbitrarily conducted; the character of the vehicle, and the rate of absorption therefrom; the possible variation in the amount of drug returning to the field of vision of the counter, due to variation in the amount of urine retained in the bladder of each of the individual animals studied; and other variables. While these variables do not deter from the essential validity of the foregoing presentation they do influence slightly the absolute value of the absorption half-lives obtained by analysis of these results.

Therefore, it appears more logical to analyze our data on the basis of the initial portion of the curve, where each of the above-discussed concomitant events are yet insignificant in their effect; as is shown, for example, in the lower set of curves in Fig. 3.

SUMMARY

1. A micro method is reported for the preparation of I-131 labeled iodoform, and 2,4,6-triiodophenol at a level of activity of approximately 150 to 200 $\mu\text{c./mg.}$

2. A highly reproducible animal procedure was developed for the continuous external

detection of a gamma-emitting dose in the rectum of an anesthetized rat.

3. A mathematical expression was derived which corrects the activity detected by the counter for the effect of diffusion within the vehicle, and for the accumulation of the absorbed drug in the scanned volume segment of the animal.

4. Diffusion of the drug to the rectal absorption site appears to be the rate controlling step in fast absorbing systems.

REFERENCES

- (1) Lyman, R. A., and Sprowls, J. B., Editors, "American Pharmacy Textbook of Pharmaceutical Principles, Processes, and Preparations," 4th Ed., J. B. Lippincott Co., Philadelphia, 1955, p. 347.
- (2) Giacomini, G., and Coriandoli, E. M., "Somministrazioni dei Farmaci per via Rettale. Tecnica di Preparazione delle Forme Farmaceutiche," Gatti Editore, Milano, Italy, 1954.
- (3) Bucher, K., *Helv. Physiol. Acta*, **6**, 821(1948).
- (4) Gross, H. M., and Becker, C. H., *THIS JOURNAL*, **42**, 90(1953).
- (5) Buchi, J., and Schlumpf, R., *Pharm. Acta. Helv*, **19**, 171(1944).
- (6) Ward, W. C., *THIS JOURNAL*, **39**, 265(1950).
- (7) Gross, H. M., and Becker, C. H., *ibid.*, **42**, 96(1953).
- (8) Peterson, C. F., and Guida, A. J., *ibid.*, **42**, 537(1953).
- (9) Hassler, W. H., and Sperandio, G. J., *ibid.*, *Pract. Pharm. Ed.*, **14**, 26(1953).
- (10) Delay, J., and Thuiller, J., *Science*, **117**, 57(1953).
- (11) Cacchillo, A. F., and Hassler, W. H., *THIS JOURNAL*, **43**, 683(1954).
- (12) Middendorf, L., *Munch. med. Wochschr.*, **86**, 95(1939).
- (13) Buchi, J., and Oesch, P., *Pharm. Acta. Helv*, **20**, 129(1945).
- (14) Waxler, S. H., and Schack, J. A., *J. Am. Med. Assoc.*, **143**, 736(1951).
- (15) Canals, E., Marignan, R., and Cordier, S., *Ann. pharm. franc.*, **9**, 318(1951).
- (16) Canals, E., Marignan, R., and Cordier, S., *ibid.*, **10**, 38(1952).
- (17) Cremer, H. D., Hennig, W., and Weher, H., *Arzneimittel Forsch.*, **3**, 448(1953).
- (18) Peterson, C. F., Lee, C. O., and Christian, J. E., *THIS JOURNAL*, **42**, 713(1953).
- (19) Carbalho, L. S., and Santos, M. De L. A., *Rev. port. farm.*, **5**, 173(1955).
- (20) Hofmann, H., and Hornbogen, U. H., *Pharm. Zentralhalle*, **89**, 369(1950).
- (21) Caronnet, R., Chevallard, L., and Giono, H., *Ann. pharm. franc.*, **7**, 627(1949).
- (22) Lechat, P., and Boissire, J., *ibid.*, **13**, 683(1955).
- (23) Ballard, B., and Goyan, F. M., *THIS JOURNAL*, **47**, 40(1958).
- (24) Vogel, A. I., "A Textbook of Practical Organic Chemistry," 3rd ed., Longmans, Green and Co., Inc., New York, N. Y., 1956, p. 299.
- (25) Marsh, J. B., *J. Chem. Soc.*, **128**, 3164(1927).
- (26) Riegelman, S., and Crowell, W. J., *THIS JOURNAL*, **47**, 115(1958).
- (27) Dunwald, H., and Wagner, C., *Z. Physik. Chem.*, **B24**, 52(1934).
- (28) Johnson, F. H., Eyring, H., and Polissar, M. J., "The Kinetic Basis of Molecular Biology," John Wiley and Sons, Inc., N. Y., 1954.

The Kinetics of Rectal Absorption II.*

The Absorption of Anions

By SIDNEY RIEGELMAN and WILFRED CROWELL†

The rectal absorption rates of sodium iodide and the sodium salt of 2,4,6-triiodophenol in suppository bases at various pH values and in the presence of surface-active agents and polyethylene glycol additives were determined by continuous external detection at the site of insertion. The rate of sodium iodide absorption is accelerated in the presence of surfactants and appears to be proportional to the surface tension lowering and the peptizing action of their surface active components. The rate of absorption of the phenolate is retarded by the presence of surfactants. Hydrolysis and subsequent solubilization of the free phenol is postulated to explain this effect.

IN THE FIRST PAPER of this series (1), a method (using a female rat) was reported for the direct and continuous external detection of the rate of rectal absorption of drugs from suppository vehicles. The rate of absorption of sodium iodide, iodoform, and 2,4,6-triiodophenol tagged with iodine-131 could be followed by virtue of the gamma emission from the radioactive source which was detected by a scintillation counter in conjunction with an autoscaler. The absorption data appeared to follow a pseudo-first order rate process when corrected for the effect of two concomitant events. The drug is continuously returning to the environments of the absorption site and is detected there by the counter. Also within the confined rectal segment to which the suppository was limited, the rate limiting step in the overall absorption process is assumed to be the diffusion of the drug within the vehicle. An equation was presented which rectified the data for analysis to conform to a pseudo-first order rate process. An alternative to the use of the corrected data is the analysis of the initial portion of the uncorrected data where each of the factors causing deviation would be at a minimum. Analysis of the initial data probably results in an absorption half-life which is slightly too long, due to the small amount of curvature present even in the earliest points. While the absolute values of the specific rate constants may differ with each method of analysis, the relative comparison of the data by either method results in identical conclusions. For this reason, the simpler method of analysis using the initial data has been adopted in this report.

In the past several years, studies have been conducted (2, 3) which report on the absorption of sodium iodide from common type suppository bases. One such study (3) has utilized radioiodide as the means of detection. The authors based their conclusions on results of analysis of peripheral level of iodide in the blood, thyroid, or in the urine. While certain information can be obtained by these methods, they appear to be poorly reproducible and to give little indication of the details of the rate process directly at the site of insertion. Apparently no studies have been conducted on the effects of surface-active agents on the rate of rectal absorption, even though several nonionic surface-active agents have been utilized as major constituents of several suppository bases (4, 5).

Polyethylene glycols have been utilized quite extensively in commercial suppositories. Yet, inconsistent and contradictory results (6-10) have been obtained from studies comparing their rate of drug release to that obtained by cocoa butter or by glycerinated gelatin. Once again, these studies were restricted to methods of analysis which detect events distant from the site of absorption; thus the conclusions drawn from these results may be clouded by the number of intervening biological processes. Furthermore such studies would probably be unable to detect differences caused by complexing of the drug with the polyoxyethylene chain, as has been proposed by Higuchi and Lach (11).

The present communication presents the results of the evaluation of the absorption rate of sodium iodide (NaI) and the sodium salt of 2,4,6-triiodophenol (NaTIP) at various pH values and in the presence of surface-active agents and polyethylene glycol additives.

RESULTS AND DISCUSSION

Both sodium iodide and triiodophenol can exist as anions at certain hydrogen ion concentrations.

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Sodium iodide exists as an anion at all pH values which can be reasonably studied in the rectum and possesses high water solubility. In contrast, triiodophenol is a weak acid of very low solubility. The acid dissociation constant of the compound was reported by Kralil and Clowes (12) and was confirmed by the authors to be 6.6. The solubility of the compound in an unbuffered solution containing 5% of polyethylene glycol 600 was found to be 300 mcg. per 100 ml. At pH 8.6, triiodophenol would be distributed 99.01% as the anion and 0.99% as the free acid. An isotonic, liquid polyethylene glycol 6000 vehicle (LPEG) containing 59% of a purified sample of polymer was used in these studies to eliminate the effects of such variables as: water transfer due to the nonisotonic nature of polyethylene glycol suppositories, and/or the solution of the solid phase by the transferred water. Due to the large molecular weight of the glycol, one can assume that the compound is very slowly, if at all, absorbed; therefore, the isotonic vehicle can be expected to remain undiluted during the period of study. The dissociation of the triiodophenol in such a semiaqueous solvent system will be reduced relative to an aqueous system. The dissociation constant was found to be 8.4 in this vehicle. In order to convert the free acid to the same proportion of anion as used in the aqueous systems, this vehicle was adjusted to pH 10.7 with a sodium hydroxide-sodium borate buffer.

The results obtained with sodium iodide in the vehicles studied are summarized in Table I and several of these results are depicted in detail in Fig. 1. As one might anticipate, at the same level of viscosity, a change from methylcellulose to carboxymethylcellulose does not appreciably alter the rate of absorption of sodium iodide. It was necessary to substitute the latter agent in several of the vehicles studied due to the coacervation of methylcellulose in the presence of certain surface-active agents.

TABLE I.—COMPARISON OF THE FIFTY PER CENT ABSORPTION TIMES OF VEHICLES CONTAINING SODIUM IODIDE

Type	Vehicle	Surface Tension (dynes/cm)	50% Absorption Times ($t_{1/2}$)
MC	Plain	52	33.1 \pm 0.8
CMC	Plain	52	31.4 \pm 0.5
LPEG	Plain	46	29.5 \pm 1.3
MC	Tw20-1%	34	21.5 \pm 0.6
MC	Tw20-5%	34	22.8 \pm 0.6
CMC	Tw20-1%	33	22.2 \pm 0.4
CMC	SLS-1%	30	8.9 \pm 0.2
CMC	A400-1%	30	8.4 \pm 0.3

The addition of surface-active agents to these systems markedly accelerated the rate of absorption of the sodium iodide when compared to the rate in their absence. Surface-active agents are frequently said to cause protein denaturation and may actually damage the integrity of the mucous membrane lining of the rectum. Such an explanation might be presented for the increased rate of absorption of iodide ions in the presence of surfactants. Yet, identical studies conducted on sodium triiodophenolate (NaTIP) in the presence of the same group of surf-

actants indicate that this ion is actually retarded in its rate of absorption relative to the rate in the absence of surface-active agents. It is difficult to visualize how the surfactant can damage the mucous membrane in such a way that iodide ions can penetrate, yet not cause an increased rate of absorption of the phenolate ion.

Since the segment from which absorption is taking place is held constant by the confining end plugs, the action of the surface-active agents on the rate of drug absorption cannot be attributed to the spreading of the vehicles over a larger rectal segment. The observed differences in time for 50% absorption of NaI in the presence of Tween® 20 (Tw20), sodium lauryl sulfate (SLS) and Antarox® A400 (A400)¹ appear to correlate with the relative

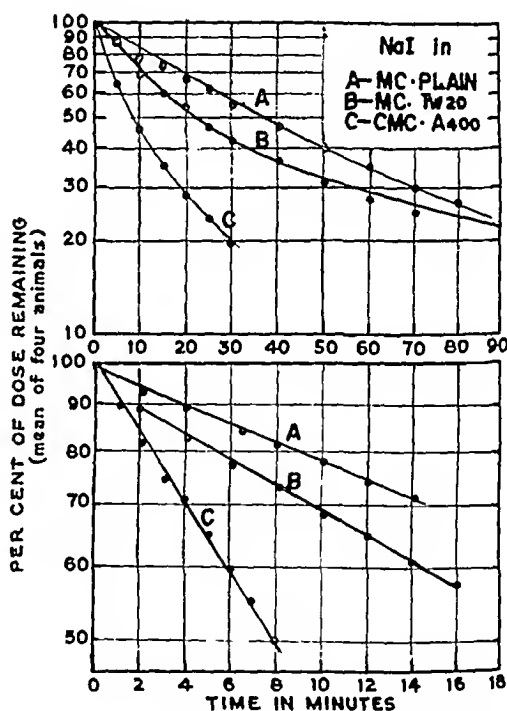


Fig. 1.—Rectal absorption curves for sodium iodide from three suppository vehicles: MC-plain, MC-Tw20-1%, and CMC-A400-1%. The upper curves represent the total period of observation; the lower curves represent the initial eighteen-minute portion of the observed data.

surface tension lowering of the respective vehicles. Since the rectal membrane is covered with a relatively continuous mucous blanket, the acceleration of NaI absorption might well be ascribed to the mucous peptizing action of the vehicle ingredients. As a result of this cleansing action, additional pore spaces could be made available to the iodide ions, thus facilitating their movement across the membrane barrier.

It is interesting to note the data obtained with sodium iodide dissolved in the LPEG vehicle are also in accord with the above peptizing theory.

¹ This compound is a polyethylene glycol ether of nonyl phenol and is now sold under the trade name of Igepal® CO-630.

The cleansing or peptizing capacity of surfactants is not well represented by comparison of the reduction in surface tension of the solution. Several physical tests are required to evaluate the deterative activity of surfactants (13), however, such studies indicate that Tw20 is less effective than SLS or A400. The latter two agents appear to be equally effective in their detergent action. A similar relationship was found in the relative effectiveness in modifying the rate of absorption of NaI.

No doubt, without further experimentation, any theory put forward to explain the effect of surfactants will be controversial; nevertheless the fact remains that the addition of small amounts of these additives strikingly increases the rate of absorption of the iodide ion from the formulations studied. It appears that they accelerate the rate fourfold to fivefold.

Table II and Fig. 2 appropriately summarize the data obtained for NaTIP in the suppository systems studied. Once again the data confirm the postulate that methylcellulose and carboxymethylcellulose are interchangeable in the vehicles with little or no effect on the rate of absorption. Thirty-two milligrams of NaTIP were present per 100 ml. of the vehicles. Therefore at pH 8.6, where 99% of the compound was in the anion form, the vehicle approached saturation in respect to the free acid (HTIP). At pH 10, however, only 0.03% of the total drug present was in the free acid form, making the vehicle approximately 3% saturated. Even though there is a thirtyfold change in HTIP concentration between 8.6 and 10.0, a relatively insignificant change occurs in the absorption half-life of the drug.

TABLE II.—COMPARISON OF THE FIFTY PER CENT ABSORPTION TIMES OF VEHICLES CONTAINING SODIUM TRIIODOPHENOLATE

Type	Vehicle Additive	Surface Tension (dynes/cm.)	50% Absorption Times (t _{1/2})	pH
MC	Plain	52	17.3 ± 1.4	8.6
CMC	Plain	52	18.6 ± 0.2	8.6
MC	Plain	55	19.9 ± 0.4	10.0
LPEG	Plain	46	42.6 ± 0.4	10.7
CMC	SLS-0.25%	33	24.2 ± 0.3	8.6
CMC	SLS-1%	33	59.3 ± 1.7	8.6
MC	Tw20-1%	34	40.3 ± 1.2	8.6
MC	Tw20-5%	34	41.8 ± 0.8	8.6

The time for 50% absorption of NaI in the absence of surfactants is significantly greater than that for NaTIP even though the molecular volume of the iodide ion is appreciably smaller than the phenolate ion. The reverse might have been expected since both the diffusion and absorption processes are known to be related to molecular volume characteristics. Theoretically, there are several factors which may account for the rate differences. Even though the absorption of NaI possesses first-order characteristics over a short range of concentrations, its absorption might be sufficiently altered to give a slightly shorter half-life if used in reduced concentrations. A more plausible suggestion is that a portion of the phenolate ion is converted into the free phenol at the absorption site during the transfer of ions be-

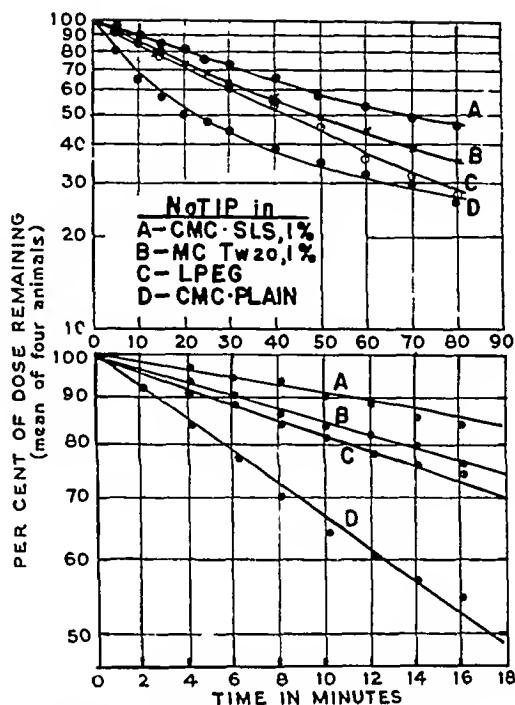


Fig. 2.—Rectal absorption curves for sodium triiodophenolate from four suppository vehicles: CMC-SLS-1%, MC-Tw20-1%, LPEG, and CMC-plain. The upper curves represent the total period of observation; and the lower curves represent the initial eighteen-minute portion of the observed data.

tween intracellular and extracellular fluids. Since both HTIP and the NaTIP ion might be absorbed independently, the observed absorption rate may be the sum of the individual absorption rates.

While surface-active agents accelerate the absorption of NaI, the data presented in Table II and in Fig. 2 clearly indicate that the reverse is found for NaTIP systems. One explanation of the slow rate of absorption might be related to the observation of Higuchi and co-workers (11), who have pointed to the complexing of certain phenols with long chain polyoxyethylene polymers. One might conclude from their work that either the phenolate ion or the free phenol complexes with the polyoxyethylene moiety in the micelles, or directly as in the case of the LPEG vehicles.

In contrast to Tw20 and A400, SLS possesses no polyoxyethylene chains, but instead has an anionic polar sulfate group; nevertheless, it markedly retards NaTIP absorption. Since micelles are present in the SLS systems studied, solubilization of the drug within the hydrocarbon portion of the micelle might be postulated. This kind of solubilization would require interaction of the anionic triiodophenolate ions with the anionic surfactant ions. Such an occurrence is unlikely since it would be opposed by the coulombic repulsive forces between these ions. However, the surfactant micelle could solubilize the undissociated triiodophenol.

In the presence of surface-active agents, the following equation may indicate the interrelationship of the surfactant to the triiodophenol:

equivalent for the first eight minutes of each experimental series; for slow absorption, for all other absorption studies, on data collected for the first eighteen minutes. Time for 50% of the dose to be absorbed from each vehicle was determined by substituting the values of the slope and standard error of the slope into the following equation:

$$t^{1/2} \text{ (in mins.)} = 0.301/(\text{slope} \pm 0.301/\text{standard error of the slope}) \text{ (Eq. 2)}$$

Solubilization Studies.—The solubilization of TIP in various aqueous systems was determined by the following nephelometric procedure. Approximately 100 mg. of the compound was dissolved in sufficient PEG 600 to make a stock solution containing 1 mg. of solute per 1000 mg. of solvent. Aliquots of the stock solution were weighed into 100-ml. tared volumetric flasks and then diluted to volume with isotonic solutions or isotonic buffered solutions containing the surface-active agent. After mixing, the degree of light scattering for each system was detected with a Fischer Nefluoro-Photometer and the per cent transmission was plotted against solute concentration. By extrapolation of the plotted points to solvent transmission, a numerical value was obtained for the amount of solute solubilized in each system.

Determination of the Apparent Dissociation Constant of Triiodophenol in PEG Vehicles.—The de-

termination was carried out at room temperature using a Beckman pH meter, Model G, and a 1-ml. semimicro buret. Five-milliliter aliquots of PEG vehicle containing 0.02 millimole of the free acid were titrated to neutrality with 0.1N NaOH in an identical PEG vehicle. The pH readings obtained from this procedure were plotted on centimeter paper against the microliters of alkali added. From the titration curves thus constructed, the pK was evaluated to be 8.4 ± 0.1 .

REFERENCES

- (1) Riegelman, S, and Crowell, W. J, *THIS JOURNAL*, **47**, 115(1958).
- (2) Büchi, J., and Oesch, P, *Pharm Acta Helv.*, **20**, 129 (1945).
- (3) Peterson, C. F., Lee, C. O., and Christian, J. E., *THIS JOURNAL*, **42**, 713(1953).
- (4) Ward, W. C., *ibid.*, **39**, 265(1950).
- (5) Gross, H. M., and Becker, C. H., *ibid.*, **42**, 96(1957).
- (6) Middendorf, L., *Munch med Wochschr.*, **86**, 95(1939).
- (7) Hassler, W. H., and Sperandio, G. H., *THIS JOURNAL*, *Pract Pharm Ed.*, **14**, 26(1953).
- (8) Caronnat, R., Cheviallard, L., and Giono, H., *Ann pharm frang.*, **7**, 627(1949).
- (9) Waxler, S., and Schack, J., *J. Am Med Assoc.*, **143**, 736(1950).
- (10) Truitt, E. B., Jr., McKusick, V. A., and Krantz, J. C., Jr., *J. Pharmacol Exptl. Therap.*, **100**, 309(1950).
- (11) Higuchi, T., and Lach, J. L., *THIS JOURNAL*, **43**, 465 (1954).
- (12) Krahle, M. E., Clowes, G. H., *J. Cellular & Comp Physiol.*, **11**, 1(1938).
- (13) Mankowich, A. M., *Ind Eng Chem.*, **44**, 1151(1952).
- (14) Snedecor, G. W., "Statistical Methods," 5th Ed., The Iowa State College Press, Ames, Iowa, 1956, p. 140.

The Kinetics of Rectal Absorption III.*

The Absorption of Undissociated Molecules

By SIDNEY RIEGELMAN and WILFRED J. CROWELL†

A study of the rectal absorption of iodide-131 tagged iodoform and 2,4,6-triiodophenol, at pH's where the drug is undissociated, was carried out in liquid semi-aqueous isotonic vehicles containing the drug in suspension, in true solution, and in solution in surfactant micelles. First order analysis of the results indicated that the drugs are best absorbed from true solutions in water, or from water suspensions of the drug. Surface-active agents and polyoxyethylene polymers markedly retard the rate of absorption. The relative particle size of the suspended drug influenced the absorption rate. Solution of the drugs in solid polyethylene glycol and oleaginous bases resulted in very prolonged absorption times. Head count studies (simulating continuous blood level studies) and the data on the tissue distribution of the compounds are also presented.

OIL-SOLUBLE, undissociated compounds have been used frequently as test drugs by workers studying the factors affecting the rate of absorption of drugs from suppository bases. However, probably due to the method for detection of the drug available to these workers, only conditions producing differences of relatively

large magnitude have been able to be detected. One of the most important of these methods has been the analysis of the concentration of the compound in the blood stream. While this method results in valuable information pertinent to that one drug, such studies are confounded by biotransformations of the drug and by the potential difference in the affinities of the tissues for the compound at varying rates of absorption. The results, therefore, have been very difficult to generalize to other compounds, even in the same vehicle. Unless there is a direct, proven relationship between the rate of absorption at the

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rectal site and the blood level, it is difficult to draw general conclusions. By the use of a direct radiological method of detection of the events taking place at the site of absorption, this present series of studies (1, 2) has been able to detect relatively subtle differences in the rate of absorption from suppository vehicles.

This communication reports the results obtained by studying the absorption of two radio-tagged, undissociated molecules—2,4,6-triiodophenol (HTIP), and iodoform (IF) from the rectal passage of a female rat. Liquid isotonic semi-aqueous vehicles have been formulated containing the drugs in suspension, in true solution in water and in polyethylene glycol, and micellarily solubilized by surface active agents. Solid polyethylene glycol and cocoa butter type bases have been studied in an attempt to correlate such data with the results of other workers in the field. In addition, this communication includes data on the total tissue distribution of the drugs and head counts (simulating continuous blood level studies) on the two undissociated molecules as well as sodium iodide. It is now possible to draw some comparisons and generalizations from the studies relative to the work done by other techniques.

RESULTS AND DISCUSSION

It is essential for these studies that extraneous iodide ¹³¹I be removed or reduced to an absolute minimum in the samples of iodoform and triiodophenol. During the synthesis of the compounds, several additional steps were taken to remove all traces of iodide ¹³¹I. The purity of both compounds was evaluated by paper chromatography combined with radioautographic techniques. Radio-tagged iodoform samples were found to contain one to two% radioactive iodide impurities. If the iodoform were decomposed to a large extent into iodide, the rate of absorption of the compound should follow the pattern of results obtained with that ion. The significant difference in results between the rectal absorption rates of IF and NaI ¹³¹I appears to confirm the postulate that the compound is relatively free of trace iodides and is not broken down to iodide prior to absorption. Radioactive triiodophenol was found to be essentially free of radioactive iodide impurities. Both compounds were stored in a nonaqueous solution composed of undiluted polyethylene glycol 600. This measure was necessary to prevent contamination of the area, since both compounds sublimed when stored as solids. In the majority of experiments, the radio-tagged samples were used for only a period of seven days or less in order to stay within one radioactive half-life of the iodide.

The water saturation concentration for iodoform in the plain methylcellulose vehicle was found to be 8 mg %. In the vehicles reported in Table I, iodoform was present to the extent of 32 mg %. Therefore, the compound was present as a suspended

solid phase, exceeding its solubility in the plain MC vehicle by fourfold. However, when the surface active agents were added to the MC vehicle or when the isotonic liquid polyethylene glycol (LPEG) vehicle was studied, the iodoform was completely dissolved.

TABLE I—FIFTY PER CENT ABSORPTION TIMES FOR TRIIODOPHENOL-IODOFORM FROM VARIOUS VEHICLES

Type	Vehicle Additive	50% IF Absorption Time (t _{1/2})	HTIP
MC	plain	13	35
MC	Tw 20	3 ± 0.5	0 ± 0.8 ^a
	0.25%		28
MC	Tw 20 1%	24	39
MC	Tw 20 5%	7 ± 1.2	7 ± 0.5
LPEG		25	44
		1 ± 0.4	9 ± 0.8
MC	SLS 0.25%	23	43
MC	SLS-1%	2 ± 0.4	8 ± 1.3 ^b
			49
			6 ± 0.9
			73
			3 ± 1.7

^a All MC HTIP vehicles were adjusted to pH 4.6

^b All LPEG HTIP vehicles were adjusted to pH 5.9

Triiodophenol has been found to possess a pK_a of 6.6 in water (3), and 8.4 in the LPEG vehicle. Therefore, when dispersed in a MC vehicle buffered to pH 4.6, the triiodophenol is present to the extent of 99% in the free acid form (HTIP). In the LPEG vehicle, the drug was buffered to pH 5.9 in order to assure that greater than 99% of the amount present was in the undissociated form. In the studies reported in Table I, the concentration of triiodophenol was maintained at 32 mg %. The solubility of the compound in the MC vehicle was found to be 300 mcg %, the compound, thereby, was present as a suspended phase in the MC vehicle exceeding the solubility by approximately one hundredfold. The solubility was markedly increased when the surface-active agents were added to the MC vehicle containing 32 mg % of triiodophenol. When 0.25% of sodium lauryl sulfate or Tween[®]20 were included, the HTIP only slightly exceeded the saturation concentration of the two vehicles, producing a slight amount of turbidity. The compound was completely in solution in all the other vehicles included.

In Fig. 1, curve C represents IF in the plain MC vehicle where it is in suspension, curve B, IF in solution in MC-Tw 20 1% vehicle, and curve C, IF in solution in the LPEG vehicle. Although the aqueous systems contain the drug in excess over water solubility, the rate of absorption is greater than in the Tw 20 and LPEG systems. It is interesting to observe that the rates of absorption from the two latter vehicles are very similar even though the LPEG vehicle contains many more equivalents of oxyethylene groups than the Tw 20 vehicle.

Figure 2 represents HTIP in an aqueous MC suspension—curve D, and in the presence of various concentrations of Tw 20 and SLS—curves A to C. The systems containing SLS are more effective in retarding the drug absorption than are the Tw 20 systems. A 0.25% concentration of SLS produced a slower rate of HTIP absorption than did a 5% concentration of Tw 20. The drug is micellarily solubilized by both surfactants. From the studies of Higuchi and co worker (4), we can assume interaction of the phenol with polyoxyethylene chains of Tw 20. Practically nothing is known of the type of interaction between the HTIP and the lauryl

sulfate ions; yet from the above data we must presume that the latter type of complexing is greater than the former, when evaluating on the basis of drug release.

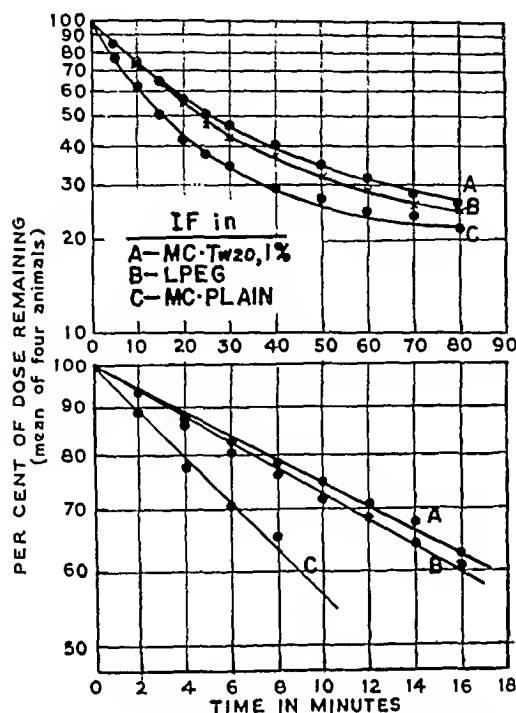


Fig. 1.—Rectal absorption curves for iodoform from three suppository vehicles. Upper curves represent total period of observation; lower curves represent initial expanded portion of observed data.

Comparison of the HTIP absorption rates at the 0.25% level of the respective SAA indicates an anomaly in the results. The 0.25% Tw 20 system caused a slight increase in the rate of absorption when compared to the aqueous suspension; while the SLS 0.25% system produced a marked retardation. Both of these vehicles contained a small excess of the drug, as compared to the aqueous MC suspension; the large increase in water solubility being due to the presence of micelles in both solutions. The rate of solution of the drug from the solid interface, as well as its rate of release from the drug-micelle complex, are undoubtedly both involved in the pattern of results. These results should be confirmed by further studies. However, the results contribute further evidence to confirm the postulate that the surface-active agents do not cause damage to the mucous membranes, which was presented in an earlier paper (2). Sodium lauryl sulfate is known to be more hemolytic than is Tw 20; indeed, the latter compound is used in parenterals. The same relative activity has been shown with other types of biological membranes. It would appear, therefore, that had the surfactants damaged the mucous membranes of the rectal passage, the rate of absorption of the drugs in sodium lauryl sulfate solution should be shorter than the comparative solution containing Tw 20. The opposite results were obtained.

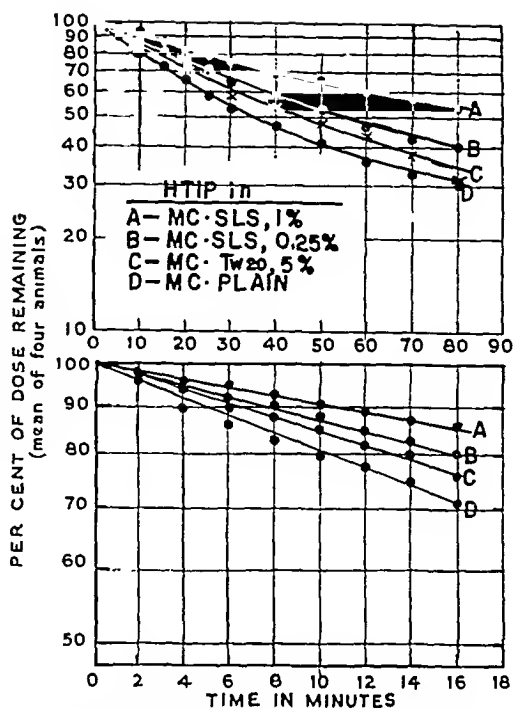


Fig. 2.—Rectal absorption curves for 2,4,6-triiodophenol in the methylcellulose vehicle and in the presence of several concentrations of two different surface-active agents. The data depict the marked retarding action of the surface-active agents.

Another interesting comparison can be made between the effects of identical concentration of surfactants on the absorption of free triiodophenol and its salt as reported previously (2). The absorption of both moieties is markedly retarded relative to systems containing no surface-active agents, as illustrated below in Table II.

TABLE II.—COMPARISON OF THE FIFTY PER CENT ABSORPTION TIMES OF TRIIODOPHENOL AND SODIUM TRIIODOPHENOLATE

Vehicle		50% Absorption Time ($t_{1/2}$)	
Type	Additive	HTIP ^a	NaTIP ^b
MC	plain	35.0 ± 0.8	17.3 ± 1.5
MC	Tw 20-1%	49.9 ± 0.8	41.8 ± 0.8
MC	Tw 20-5%	28.5 ± 0.6
MC	SLS-1%	73.3 ± 1
CMC	SLS-1%	59.3 ± 1.7
MC	SLS-5%	49.6 ± 0.9
CMC	SLS-5%	24.2 ± 0.3

^a All HTIP vehicles were buffered with an acetate buffer to pH 4.6.

^b All NaTIP vehicles were buffered with pyrophosphate to pH 8.6.

It should be noted that in the Tween systems, the time interval for 50% absorption of HTIP and NaTIP are in relatively close agreement. However, those listed for the SLS vehicles are significantly different. Anionic surfactants, such as SLS, are known to be much more affected by electrolytes than are nonionic surface-active agents, such as Tw 20. Apparently a change from a nonionic to an anionic surfactant, and a change from a mono-

valent to a polyvalent anion within the buffer greatly affects the surfactant micelle size and thereby affects the absorption rate.

Effect of Particle Size on Absorption Rate.—Both IF and HTIP were used in an attempt to evaluate the effect of particle size on the rate of rectal absorption. Data for these studies are summarized in Table III. Freshly precipitated iodoform and triio-

TABLE III.—EFFECT OF PARTICLE SIZE ON THE FIFTY PER CENT ABSORPTION TIME OF IODOFORM AND TRIIODOPHENOL

Vehcle	Concentration (mg. %)	Compound	50% Absorption Time ($t_{1/2}$)
MC	8.	IF-Soln.	9.8 ± 0.6
MC	16.	IF-Fresh ^a	13.3 ± 0.5
MC	48.	IF-Fresh ^a	29.3 ± 0.7
MC	48.	IF-Aged ^b	37.1 ± 0.6
MC	32.	HTIP-Fresh ^a	35.0 ± 0.8
MC	32.	HTIP-Aged ^b	41.6 ± 0.8

^a Precipitated in presence of methylcellulose and used within three hours after preparation

^b Precipitated in absence of methylcellulose and aged eight to ten hours before being used.

dophenol were prepared by precipitation from a solution of the compounds in polyethylene glycol 600 into an aqueous medium containing methylcellulose as a peptizing and suspending agent. This procedure produced a semicolloidal dispersion of the compounds which was stable over several days' standing. The aged systems were precipitated in the absence of methylcellulose and aged eight to ten hours prior to the addition of the methylcellulose and the use of the suppository vehicle in absorption studies. The latter procedure allowed the compounds to crystallize into distinct, large crystal masses; therefore, a large difference in surface area resulted. This can be expected to produce a large difference in the rate of solution of the two different type solids, since solution rate is directly related to surface exposed to the solvent. Radioactive HTIP was present in the above vehicles in concentrations approximately one hundred times that of water saturation. No attempt was made to study HTIP in water-saturated solution. The radioactive HTIP in the vehicles studied gave an external counting rate of 400 to 500 counts per second. It was not experimentally feasible to synthesize TIP at a higher specific activity. A water-saturated system of HTIP would have resulted in an external initial counting rate of 4 to 5 counts per second. Such a counting rate would be difficult to evaluate since it is only a few counts above normal background activity.

The IF concentrations range from saturation (8 mg. %) to approximately six times that of the water saturation value. A comparison of the different IF preparations shows that as the concentration of IF increases beyond water saturation, the rate of absorption decreases. This decrease in all probability is due to the solution of the suspended crystalline material. When the IF semicolloidal dispersions are allowed to age for twelve hours, larger crystals are formed and the absorption rate is further decreased. Similar results were obtained when these studies were repeated using freshly precipitated and aged samples of HTIP.

It is remarkable that HTIP is absorbed in such a relatively brief period of time. The experimental results show that one-half of the total amount of drug present, essentially all in the solid phase, dissolved in the vehicle and was absorbed during a thirty-five-minute period. The first paper of this series (1) presented evidence for the postulate that diffusion of the drug within the vehicle is the rate limiting step for the absorption process. It is possible that the rate of solution and, in turn, the rate of diffusion from the solid-liquid interface is the controlling factor for the pseudo-first order data obtained with these systems.

Absorption of Undissociated Compounds from Solid Vehicles.—Studies involving absorption of undissociated compounds from the rectum of the rat were also carried out using IF and HTIP in solid polyethylene glycol (SPEG), solid hard butter (SHB), and solid cocoa butter (SCB). The radioactivity was maintained at approximately the same level as used in the liquid systems. A summary of the resulting data is shown in Table IV and Figs. 3 and 4, with data for the liquid PEG vehicle included for comparative purposes.

Although their absolute solubilities were not determined, both IF and HTIP were observed to be much more soluble in PEG and oleaginous bases than in aqueous MC and CMC vehicles. In general, as much as one per cent HTIP was found to be soluble in SPEG, while less than half this amount appeared to dissolve in liquid cocoa butter and hard butter.

It is apparent in Figs. 3 and 4 that the normal shape of the absorption curve is affected during the first ten minutes by the melting characteristics of the vehicles. Experiments were conducted using supercooled, liquefied form of cocoa butter and hard butter. As may be expected, no significant differences in the rate of absorption, beyond the first ten minutes, were obtained.

TABLE IV.—FIFTY PER CENT ABSORPTION TIME FROM SOLID VEHICLES

Vehcle	Compound	50% Absorption Time ($t_{1/2}$)
LPEG	IF	23.2 ± 0.4
SPEG	IF	39.5 ± 1.4
SCB	IF	169.2 ± 9.1
LPEG	HTIP	43.8 ± 1.3
SPEG	HTIP	45.4 ± 0.9
SCB	HTIP	278.2 ± 9.6
SHB	HTIP	297.8 ± 12.8

Two different physical processes may account for the significant differences in time for 50% absorption of IF and HTIP from the liquid and solid vehicles. The latter group of vehicles soften or melt at body temperature, but the drug must partition from the oil to the water phase during the absorption process. This phenomenon presents a barrier to rapid absorption. The SPEG vehicle is a hypertonic system, and it must absorb water from the rectum for its dispersion (m. p., 52–55°) and eventual release of medicaments. HTIP is absorbed from SPEG at approximately the same rate as from LPEG, while there is a significant difference in time for 50% IF absorption from similar vehicles.

It is difficult to conclude whether the observed differences are due to the degree of complexing of the two drugs or due to the concentration of PEG in LPEG as compared to SPEG.

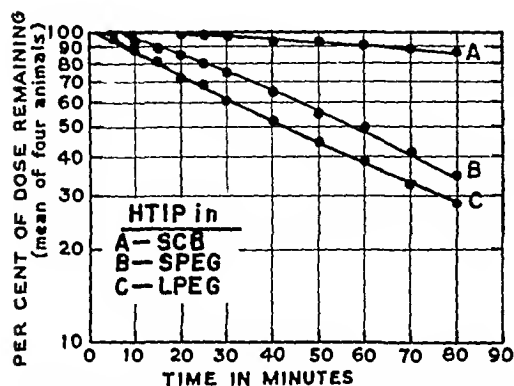


Fig. 3.—Rectal absorption curves for iodoform from a solid cocoa butter, solid polyethylene glycol and a liquid polyethylene glycol suppository vehicle.

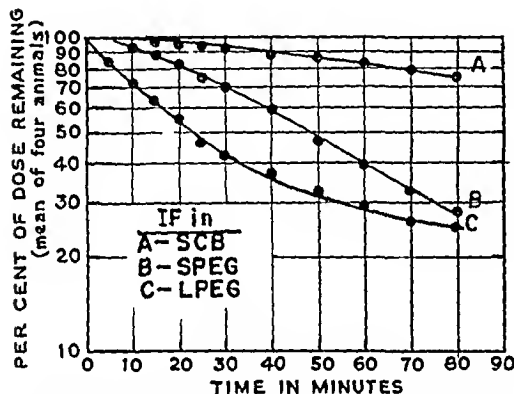


Fig. 4.—Rectal absorption curves for triiodophenol from solid oleaginous and polyethylene glycol suppository vehicles.

Head Count Studies.—A number of workers (5-9), during recent years, have endeavored to obtain kinetic data on drug absorption from the rectum by periodically sampling blood and detecting the amount of drug present. A brief study was made using an external counting method to determine the rate of accumulation of NaI-131, IF, and TIP in the area of the rat's head in an attempt to compare the rectal data obtained in the investigation with data that might be obtained by blood sampling. At the head, the level of radioactivity at any given moment should be related to the blood level of the absorbed compound.

The data obtained are represented graphically in Fig. 5. A comparison of curves A and B to curve C shows significant differences in accumulation rates of the three compounds within the head. One might arrive at two different sets of conclusions from comparison of the curves for IF and NaI-131: one set for the first twenty minutes of sampling, and a second set for the remainder of the experiment. If the results were used to interpret events taking place in the rectum, conclusions drawn

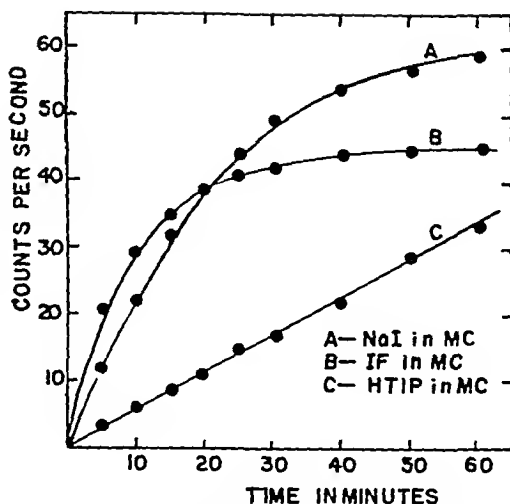


Fig. 5.—Curves showing the accumulation of rectally absorbed sodium iodide, iodoform and triiodophenol in the head of the rat.

could be very misleading since the rectal data show IF to be absorbed at a significantly faster rate than NaI-131. The head count data beyond the first twenty minutes appear to indicate that IF is absorbed from the rectum more slowly than NaI-131. The shape of the curves obtained in these studies may be related to the distribution of the absorbed compound in various tissues of the rat.

Distribution Studies.—At the end of the sixty-minute experimental head-count study, the animals were sacrificed and dissected in order to determine the distribution of the absorbed compounds in various organs and tissues. Table V summarizes the results for all three radio-tagged compounds.

TABLE V.—TISSUE DISTRIBUTION ONE HOUR AFTER RECTAL ABSORPTION OF RADIOACTIVE DOSE

Tissue	Gram Wet Weight ^a	Per Cent NaI-131 ^b	Per Cent IF ^b	Per Cent TIP ^b
Blood	3.2 ± 0.5	3.0	2.8	2.5
Heart	0.6 ± 0.1	0.4	0.4	0.6
Spleen	0.7 ± 0.3	0.5	0.6	0.7
Lungs	1.7 ± 0.3	1.0	1.1	1.3
Thyroid	0.4	0.5	0.3
Kidneys	1.4 ± 0.2	0.8	1.0	4.3
Urine	0.7	0.9	1.1
Liver	7.0 ± 0.4	2.5	3.8	9.3
Stomach	2.9 ± 1.0	7.3	10.5	2.4
Small and Large Intestine	11.8 ± 2.3	6.4	5.3	7.9
Skin	27.8 ± 2.7	15.1	14.7	13.6
Rectal Portion Containing Dose	23.5	21.8	28.9
Balance from Ground Carcass	117.0 ± 8.7	30.5	27.3	20.5
Total Dose Accounted For		92.1	90.7	93.4

^a Pooled mean and standard deviation for three groups of four animals.

^b Mean of four animals based on total dose administered.

With the exception of the kidneys, liver, ground carcass, and the rectal portion containing the dose,

valent to a polyvalent anion within the buffer greatly affects the surfactant micelle size and thereby affects the absorption rate.

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Although their absolute solubilities were not determined, both IF and HTIP were observed to be much more soluble in PEG and oleaginous bases than in aqueous MC and CMC vehicles. In general, as much as one per cent HTIP was found to be soluble in SPEG, while less than half this amount appeared to dissolve in liquid cocoa butter and hard butter.

It is apparent in Figs. 3 and 4 that the normal shape of the absorption curve is affected during the first ten minutes by the melting characteristics of the vehicles. Experiments were conducted using supercooled, liquefied form of cocoa butter and hard butter. As may be expected, no significant differences in the rate of absorption, beyond the first ten minutes, were obtained.

TABLE IV.—FIFTY PER CENT ABSORPTION TIME FROM SOLID VEHICLES

Vehicle	Compound	50%
		Absorption Time (<i>t</i> _{1/2})
LPEG	IF	23.2 ± 0.4
SPEG	IF	39.5 ± 1.4
SCB	IF	169.2 ± 9.1
LPEG	HTIP	43.8 ± 1.3
SPEG	HTIP	45.4 ± 0.9
SCB	HTIP	278.2 ± 9.6
SHB	HTIP	297.8 ± 12.8

Two different physical processes may account for the significant differences in time for 50% absorption of IF and HTIP from the liquid and solid vehicles. The latter group of vehicles soften or melt at body temperature, but the drug must partition from the oil to the water phase during the absorption process. This phenomenon presents a barrier to rapid absorption. The SPEG vehicle is a hypertonic system, and it must absorb water from the rectum for its dispersion (m. p., 52–55°) and eventual release of medicaments. HTIP is absorbed from SPEG at approximately the same rate as from LPEG, while there is a significant difference in time for 50% IF absorption from similar vehicles.

It is difficult to conclude whether the observed differences are due to the degree of complexing of the two drugs or due to the concentration of PEG in LPEG as compared to SPEG.

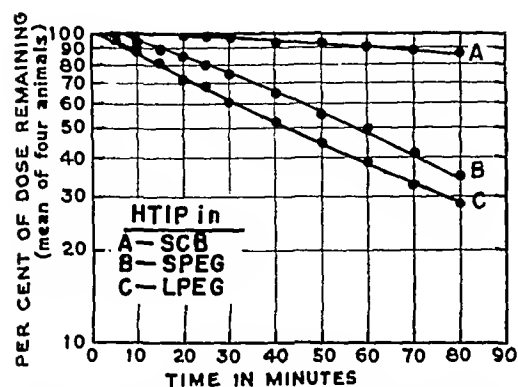


Fig. 3.—Rectal absorption curves for iodoform from a solid cocoa butter, solid polyethylene glycol and a liquid polyethylene glycol suppository vehicle.

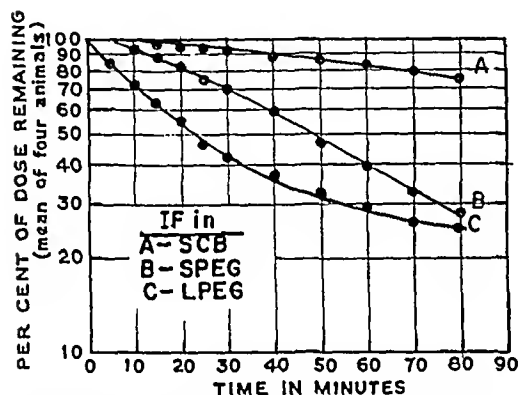


Fig. 4.—Rectal absorption curves for triiodophenol from solid oleaginous and polyethylene glycol suppository vehicles.

Head Count Studies.—A number of workers (5-9), during recent years, have endeavored to obtain kinetic data on drug absorption from the rectum by periodically sampling blood and detecting the amount of drug present. A brief study was made using an external counting method to determine the rate of accumulation of NaI-131, IF, and TIP in the area of the rat's head in an attempt to compare the rectal data obtained in the investigation with data that might be obtained by blood sampling. At the head, the level of radioactivity at any given moment should be related to the blood level of the absorbed compound.

The data obtained are represented graphically in Fig. 5. A comparison of curves A and B to curve C shows significant differences in accumulation rates of the three compounds within the head. One might arrive at two different sets of conclusions from comparison of the curves for IF and NaI-131: one set for the first twenty minutes of sampling, and a second set for the remainder of the experiment. If the results were used to interpret events taking place in the rectum, conclusions drawn

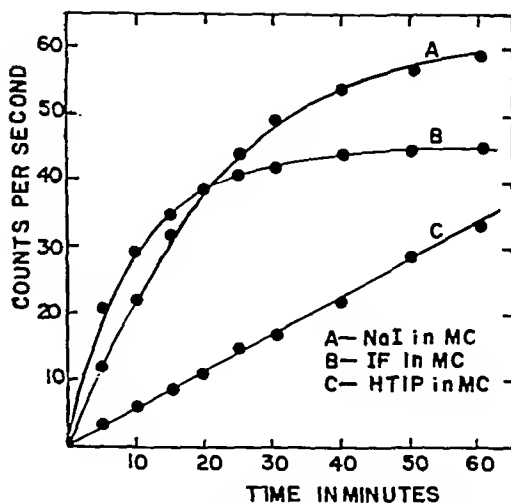


Fig. 5.—Curves showing the accumulation of rectally absorbed sodium iodide, iodoform and triiodophenol in the head of the rat.

could be very misleading since the rectal data show IF to be absorbed at a significantly faster rate than NaI-131. The head count data beyond the first twenty minutes appear to indicate that IF is absorbed from the rectum more slowly than NaI-131. The shape of the curves obtained in these studies may be related to the distribution of the absorbed compound in various tissues of the rat.

Distribution Studies.—At the end of the sixty-minute experimental head-count study, the animals were sacrificed and dissected in order to determine the distribution of the absorbed compounds in various organs and tissues. Table V summarizes the results for all three radio-tagged compounds.

TABLE V.—TISSUE DISTRIBUTION ONE HOUR AFTER RECTAL ABSORPTION OF RADIOACTIVE DOSE

Tissue	Gram Wet Weight ^a	Per Cent NaI-131 ^b	Per Cent IF ^b	Per Cent TIP ^b
Blood	3.2 ± 0.5	3.0	2.8	2.5
Heart	0.6 ± 0.1	0.4	0.4	0.6
Spleen	0.7 ± 0.3	0.5	0.6	0.7
Lungs	1.7 ± 0.3	1.0	1.1	1.3
Thyroid	0.4	0.5	0.3
Kidneys	1.4 ± 0.2	0.8	1.0	4.3
Urine	0.7	0.9	1.1
Liver	7.0 ± 0.4	2.5	3.8	9.3
Stomach	2.9 ± 1.0	7.3	10.5	2.4
Small and Large Intestine	11.8 ± 2.3	6.4	5.3	7.9
Skin	27.8 ± 2.7	15.1	14.7	13.6
Rectal Portion Containing Dose	23.5	21.8	28.9
Balance from Ground Carcass	117.0 ± 8.7	30.5	27.3	20.5
Total Dose Accounted For		92.1	90.7	93.4

^a Pooled mean and standard deviation for three groups of four animals.

^b Mean of four animals based on total dose administered.

With the exception of the kidneys, liver, ground carcass, and the rectal portion containing the dose,

all three compounds are rather uniformly distributed in the rat. The per cent discrepancy between the amount of drug administered and the sum of the individual tissue percentages is due to the differences in the geometry under which the samples are observed by the counter.

The distribution of IF in the rat parallels that of NaI-131. The iodide is known to follow chloride distribution in the body. Since chloride is confined largely to the extracellular fluids, it may be assumed that IF is degraded, following absorption from the rectum to give iodide ion as one of the end products. The fact that both I-131 and IF are concentrated in the stomach to a much greater degree than TIP would support the latter assumption.

As compared to I-131 and IF, TIP is more concentrated in the liver and kidneys of the rat, but less concentrated in the stomach tissues. The lower per cent value for TIP in the latter organ might be attributed to the stability of this compound in the presence of metabolic processes which possibly degrade IF to the iodide state.

During the NaI-131 distribution studies, a non-fasted rat was accidentally used; upon analysis, the stomach distribution value was found to be almost double that of the fasted animals. One might conclude, in view of this finding, that the blood levels are dependent on the degree of fasting of the test animal.

The amount of carrier in a vehicle influences the concentration of absorbed compound in the blood or various tissues; for example, Pearlman and co-workers (10) have found that when a tracer dose of NaI-131 containing 0.5 mg. inert potassium iodide is administered to rats, less than 2% is concentrated in the thyroid during a five-hour period. This value is in accord with our finding of 0.39%, found one hour after administering NaI-131 in the presence of approximately 0.33 mg. of inert sodium iodide.

EXPERIMENTAL

The radiological procedures and equipment, the pharmacological and counting procedures, the method of synthesis of HTIP and IF, the method of determining the radiochemical purity of HTIP, the assay of the radioactivity of the labeled compounds, and the physical characterization of the suppository vehicles have been described previously (1, 2).

Reagents.—The reagents used in this study which were not reviewed previously (1, 2) include: cocoa butter, U. S. P. grade, (Ghirardelli Co.) m. p. 34.5°; and hard butter, type M (Best Foods); a triglyceride substitute for cocoa butter possessing a softening point of 37.4°, saponification value 234, iodine value 2.9.

Formulas for Suppository Vehicles.—The basic methylcellulose (MC) vehicle used for both IF and HTIP is given below:

Polyethylene glycol 600.....	5.0 Gm.
Methylcellulose 4000 c. p. s.....	1.5 Gm.
NaCl.....	<i>q. s.</i> to make isotonic
Buffer.....	<i>q. s.</i> ad 100.0 ml.

A 0.05 *M* phosphate buffer, pH 7.4, was used for IF; while a 0.05 *M* acetate buffer, pH 4.6, was used for HTIP. The surface-active agents were added to the above formulations in the required amounts

(0.25 to 5%) and the vehicles were readjusted for tonicity and viscosity.

The isotonic liquid polyethylene glycol (LPEG) vehicle was designed to contain a large amount of purified polyethylene glycol 6000 in order to balance the viscosity and tonicity of the vehicle as well as possessing solvent properties for the IF and HTIP. The formulas for both compounds included:

Polyethylene glycol 6000.....	59.0 Gm.
Polyethylene glycol 600.....	5.0 Gm.
Distilled water (IF)	
or Acetate buffer (0.05 <i>M</i> , pH 5.9)	
.....	<i>q. s.</i> ad 100.0 ml.

The solid polyethylene glycol vehicle (SPEG) used for both IF and HTIP was formulated as follows:

Polyethylene glycol 6000.....	85.0 Gm.
Polyethylene glycol 600.....	5.0 Gm.
Distilled water to make.....	100.0 Gm.

Solid cocoa butter (SCB) vehicle and the solid hard butter (SHB) vehicle were used as received from the manufacturer.

Preparation and Insertion of Solid Vehicles.—When IF and HTIP were intended for incorporation into solid vehicles, they were dissolved individually in 3 ml. of hot ethanol. Aliquots of each ethanol solution were distributed equally among three 12-ml. centrifuge tubes. The labeled compound in each tube was precipitated from solution by the addition of distilled water and collected by centrifugation. The activity of the drug was determined and sufficient melted base was added to bring the compound into solution. While in the fused state, the vehicle containing the labeled compound was transferred in 0.2-ml. quantities, containing 5 μ c. activity, to a series of specially prepared glass tubes. Each tube was 4 cm. in length with a 2-mm. inside diameter. A piece of cork was placed midway in the empty tube to serve later as a plunger for inserting the suppository.

When the solid preparations were administered, the glass tube containing the cork-suppository unit served as a distal rectal plug. Leakage of the vehicle to the outside was prevented by placing a layer of tape around the portion of the tube introduced into the anal opening. The cork remained in the orifice of the tube after delivery of the suppository to complete the seal.

Distribution Studies.—Each IF, TIP, and NaI-131 distribution study represents the mean of the data obtained from four rats, which were sacrificed one hour after the rectal administration of a 0.2-ml. dose of labeled substance in MC vehicles. The per cent of the dose in various tissues was determined by skinning each animal, dissecting its organs, and grinding up the remains in a meat grinder. The skin and ground portions were put in separate round paper containers while the organs were placed on small round tin planchets. In order to correct the geometry and radioactive decay, an aliquot portion of the dose was run on the same shelf, in a similar planchet, and at the same time as the tissues. From the aliquot portion (1 ml.) of the diluted dose (0.2 ml. *q. s.* ad 100 ml.) the total number of counts per second for the dose administered was calculated. The activity of the labeled compound in each organ

was then expressed as a percentage of the calculated value. A second scintillation counter was used for this procedure.

Head Count Studies.—Each IF, TIP, and NaI-131 head count study represents the mean of the data obtained from four rats. Each rat was prepared in the same manner as described for the rectal studies using MC vehicles with the following exception—the hind legs of the rat were taped to the plexiglass with the upper extremities extending over the edge of the plexiglass support so as to allow the head of the rat to cover the one-inch collimator opening. This type of placement allowed one to duplicate the geometry more accurately than other methods attempted. The collimator was shielded with lead bricks so that initial counts were at background level. Instead of following the rate of disappearance of each labeled compound from the rectum, its rate of accumulation was determined in

the head of the animal at five-minute intervals for the first hour.

REFERENCES

- (1) Riegelman, S, and Crowell, W J, *THIS JOURNAL*, 47, 115(1958)
- (2) Riegelman, S, and Crowell, W J, *ibid*, 47, 128(1958)
- (3) Krah, M E, and Clowes, G H, *J. Cellular Comp Physiol*, 11, 1(1938)
- (4) Higuchi, T, and Lach, J L, *THIS JOURNAL*, 43, 465 (1954)
- (5) Waxler, S H, and Schack, J A, *J. Am Med Assoc*, 143, 736(1950)
- (6) Canals, E, Marignan, R, and Cordier, S, *Ann pharm franc*, 9, 318(1951)
- (7) Canals, E, Marignan, R, and Cordier, S, *ibid*, 10, 38(1952)
- (8) Cremer, H D, Hennig, W, and Weber, H, *Arzneimittel-Forsch*, 3, 448(1953)
- (9) Peterson, D F, Lee, C O, Christian, J E, *THIS JOURNAL*, 42, 713(1953)
- (10) Perlman, I, Chalkoff, I L, and Norton, M E, *J Biol Chem*, 139, 433(1941).

Colorimetric Determination of Thymol in Thyme Oil*

By L. FIBRANZ, M. I. BLAKE, and C. E. MILLER

A method for the analysis of thymol in thyme oil is presented. It is based upon the absorptive properties of the yellow color which develops when thymol is treated with *p*-dimethylaminobenzaldehyde in sulfuric acid as solvent. Both the red and white varieties of thyme oil are investigated. The procedure is compared with the official method and a nonaqueous titration method.

THYME OIL, typical of the volatile oils which contain predominantly phenolic constituents, is assayed by treatment with an alkali hydroxide (1). According to Guenther (2) this technique was first applied by Gildemeister for the determination of phenols in thyme oil. It is now a general method for the analysis of phenols in volatile oils.

Guenther points out several factors which may lead to erroneous results. Any alkali-soluble or water-soluble constituents or adulterants will dissolve in the aqueous phase and will be calculated as phenols. An aqueous solution of alkali phenoxide is a better solvent for the nonphenolic portion of an oil than is the alkali solution. The use of large sample volumes (10 ml.), the difficulty of reading the meniscus accurately, and the lengthy reaction period (overnight for thyme oil) are additional drawbacks. Experimental work in this laboratory indicates that good precision is

not attainable with this assay procedure.

Menthol in peppermint oil has recently (3) been accurately determined by measuring the absorptive properties of the red color which develops when menthol is treated with *p*-dimethylaminobenzaldehyde. The present report describes a similar reaction for thymol. The method is applied to the quantitative determination of thymol in thyme oil and is compared with the official procedure and a nonaqueous titration method.

EXPERIMENTAL

Color Reagent.—The color reagent was prepared by dissolving 125 mg. of *p*-dimethylaminobenzaldehyde, reagent grade, in 100 ml. of concentrated sulfuric acid, reagent grade. Fresh reagent was prepared daily.

Standard Curve for Thymol.—A standard thymol solution was prepared by weighing 55.0 mg. of N. F. thymol into a 50-ml. volumetric flask. Chloroform, U. S. P. grade, was added to the mark. Exactly 1.0 ml. of the standard solution was transferred by pipet into a 25-ml. volumetric flask and the flask was diluted to the mark with chloroform. Each milliliter of the second dilution contained 44.0 μ g. of thymol. A standard curve was prepared from aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml. of the second dilution. This represented a concentration range for thymol of 8.8 to 44.0 μ g. The aliquots were transferred to 50-ml. glass-stoppered graduates. Exactly 5.0 ml. of color reagent was added from a buret to each cylinder. The cylinders were stoppered and shaken briskly for about ten seconds

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to hasten the color reaction. A bright yellow color developed immediately in each cylinder varying in intensity with concentration of thymol. The colored solutions were transferred to colorimeter tubes and readings were taken with the Klett-Summerson photoelectric colorimeter using the blue filter No. 42 (400-465 $m\mu$). An average of three readings was recorded for each of the solutions. One milliliter of chloroform and 5.0 ml. of color reagent were treated in the same manner as the thymol solutions and served as the blank. Scale readings were directly proportional to the concentration of thymol and the absorbance of the colored solution. A standard curve for thymol was constructed by plotting scale readings vs. thymol concentrations on ordinary graph paper. The range of concentrations used in this study obeyed Beer's law.

Color Development.—In a preliminary study an attempt was made to construct a standard curve using *p*-dimethylaminobenzaldehyde T. S., U. S. P. (4), as the color reagent. This solution contains 35 ml. of water, 65 ml. of sulfuric acid, and 125 mg. of *p*-dimethylaminobenzaldehyde in each 100 ml. Although excellent results were reported for menthol (3), reproducible results were not obtained with thymol in the present study. Standardizing the conditions for color production was next considered. Each aliquot of standard thymol solution was treated in the same manner with color reagent and all were shaken simultaneously by means of an International Bottle Shaker for a specific length of time. The color intensities were noted after a definite time period. Repeated runs were conducted varying the shaking time from five minutes to twenty minutes, and the overall time period for color development was varied from ten minutes to two hours. Satisfactory results were not obtained. The composition of the color reagent was then investigated. In another series of tests the relative concentration of acid and water was varied. When concentrated sulfuric acid was used as the solvent for the color reagent, constant and reproducible results were obtained. Uniformity of shaking and the time factor were of little importance. The same concentrations used for establishing the calibration curve were employed for this study. After the color reagent was added to the aliquots of standard thymol solution, the cylinders were shaken by hand for about ten seconds. The color intensities were then read immediately, after a five-minute waiting period; and subsequently, after fifteen-minute time intervals, for a total time period of two hours. Scale readings after the first series varied less than 2% from the original readings.

Analysis of Thyme Oil.—Oil of thyme was placed into a two-dram dropper bottle and the system was weighed. The white (commercial grade) and red (N. F. VII) varieties of thyme oil were used in this study. Approximately 100 mg. of oil (3 to 4 drops), accurately weighed, was removed from the dropper bottle and placed into a 50-ml. volumetric flask. The flask was filled to the mark with chloroform. One milliliter of this solution was transferred by pipet into a 25-ml. volumetric flask and the second flask was subsequently filled to the mark with chloroform. The concentration of thyme oil in the second flask was approximately 80.0 $\mu\text{g./ml.}$ or about twice the concentration of the standard

thymol solution used to prepare the standard curve. Exactly 1.0 ml. of the second dilution was transferred by pipet into a 50-ml. glass stoppered cylinder and was treated with color reagent as described previously for the preparation of the standard curve. Three additional 1.0-ml. aliquots were treated similarly. The concentration of thymol was determined by applying the photometer reading to the standard curve. The average for the four aliquots was calculated. The precision was better than 1 part per 1,000. Four separate analyses were run on both the red and white thyme oils. The results are reported in Table I. The second column of the table

TABLE I.—COLORIMETRIC DETERMINATION OF THYMOL IN THYME OIL

Assay No.	Thyme Oil Concn. ^a ($\mu\text{g./ml.}$)	Thymol Concn. ^a ($\mu\text{g./ml.}$)	Phenol, ^b %
Red Variety			
1	82.4	23.7	28.76
2	81.7	23.6	28.89
3	82.3	23.3	28.31
4	80.1	23.2	28.96
		Av.	28.73
		Av. Dev.	0.21
White Variety			
1	77.3	16.6	21.47
2	77.3	16.8	21.73
3	79.6	17.0	21.36
4	80.3	17.6	21.92
		Av.	21.62
		Av. Dev.	0.23

^a Concentration per ml. of second dilution.

^b Determined as thymol.

indicates the concentration of thymol in each ml. of the second dilution. Column three shows the thymol concentration as determined from the standard curve and represents the average of four samples run concurrently. The phenol percentage of the oil, determined as thymol, is listed in the fourth column.

Interfering Substances.—Of the substituents normally occurring in thyme oil as recorded by Guenther (5), linalool, α -pinene, *p*-cymene, geraniol, and borneol, all in high concentrations, produce color with the reagent. For each constituent a standard solution was prepared as described earlier for thymol, except that the concentrations were about one-half that of the thymol standard solution. These dilutions represented concentrations of the constituents many times those in which they normally appear in thyme oil. In each instance the intensity of color as recorded by the photometer was not significant. It is conceivable, however, that if any one of these constituents were present in the oil in unusually large amounts, it would be possible for it to interfere with the analysis.

Official Assay of Thyme Oil.—Both varieties of thyme oil were assayed as directed in N. F. X (1). The results are shown in Table II.

Nonaqueous Titration of Thyme Oil for Phenol Content¹—Preparation of 0.1 N Sodium Methoxide.—To 100 ml. of absolute methanol, reagent grade,

¹ More complete details of this phase of the work will be reported by one of the authors (M. I. B.) in a subsequent publication dealing with the analysis of phenol-containing volatile oils.

approximately 5 Gm. of clean sodium was added slowly while the mixture was immersed in an ice bath. After all the sodium dissolved an additional 150 ml. of methanol was added, followed by 1,500 ml. of dry benzene. The solution was stored in the

TABLE II.—OFFICIAL ASSAY OF THYME OIL

Assay No.	Red Thyme Oil, % Phenols	White Thyme Oil, % Phenols
1	28.5	21.4
2	29.5	21.5
3	29.0	22.0
4	28.9	22.7
Av.	29.0	21.9
Av. Dev.	0.3	0.4

reservoir of an automatic buret protected from moisture and carbon dioxide in the atmosphere. The normality of the solution was determined by standardization against reagent grade benzoic acid.

Assay Procedure.—Approximately 600 mg. of thyme oil, accurately weighed, was transferred from the dropper bottle into a 150-ml. beaker. About 30 ml. of N,N-dimethylformamide was added. The solution, magnetically stirred, was titrated with 0.1 N sodium methoxide solution using a Fisher titrimeter equipped with a sleeve-type calomel electrode and a platinum electrode. The end point was determined by plotting millivolts vs. milliliters of titrant. The data are shown in Table III. The phenol content, determined as thymol, was calculated from the expression:

$$(\text{ml.} \times N \times 150.21 \times 100) / \text{sample weight} = \% \text{ phenols (thymol)}$$

where ml. = milliliters of sodium methoxide solution, N = normality of sodium methoxide solution, 150.21 = mEq. weight of thymol, and sample weight = weight of sample in mg.

TABLE III.—NONAQUEOUS TITRATION OF THYME OIL

Assay No.	Red Thyme Oil, % Phenols	White Thyme Oil, % Phenols
1	28.65	21.25
2	29.01	21.81
3	29.11	21.93
4	28.58	21.55
Av.	28.84	21.64
Av. Dev.	0.22	0.24

RESULTS AND DISCUSSION

The many shortcomings of the official assay procedure for determining phenols in volatile oils prompted the investigation for an improved method of analysis. The procedure described in this report is based upon a method presented earlier (3) for the estimation of free menthol in peppermint oil. It was necessary, however, to modify the color reagent since *p*-dimethylaminobenzaldehyde T. S., U. S. P., did not give constant and reproducible results. The thymol concentrations employed in this study followed Beer's law when the color reagent was prepared with undiluted sulfuric acid as solvent. Both the red and white varieties of thyme oil were

studied. The results are reported in Table I. For the white thyme oil an average thymol content of 21.62 ± 0.20 is shown and for the red variety the thymol content is 28.73 ± 0.21 . According to N. F. X (1), thyme oil must contain not less than 40% phenols to meet official specifications. However, the thyme oil used in this investigation was labeled N. F. VII. According to that edition of the N. F. (6), the phenol content of thyme oil must be not less than 20%. The white thyme oil used in this study was a commercial variety and corresponded to N. F. VII standards.

The colorimetric method described in this paper has a number of advantages. Once the standard curve has been established, the routine analysis of thyme oil can be effected in less than an hour, whereas the official assay requires an overnight waiting period. Sample weights of less than one gram are needed in place of the 10-ml. for the official method. Results are thus expressed in terms of percent weight in weight and not volume in volume. The former is the usual manner for expressing the percentage strength of constituents. Greater accuracy and precision are attainable. Inaccuracies which result from the difficulty of reading the meniscus and from the solubility of nonphenolic alkali-soluble impurities present no problem.

The possibility of interfering substituents cannot be overlooked. Several of the components of thyme oil produce color with the reagent. The concentration of these constituents is normally so small in comparison to the thymol content that interference is negligible. To substantiate this fact the proposed assay procedure was compared with the official assay. Comparable results were obtained (Tables I and II). Further supportive evidence was obtained by comparison with a nonaqueous titration method. Similar results are reported (Table III). This last method will be treated more fully in a subsequent publication.

SUMMARY AND CONCLUSIONS

1. A method for the analysis of thymol in thyme oil is presented. It is based upon the color reaction between thymol and *p*-dimethylaminobenzaldehyde. Concentrations of thymol ranging from 8.8 to 44.0 $\mu\text{g./ml.}$ obey Beer's law.
2. Quantitative results are presented for the analysis of red and white varieties of thyme oil. The possible interference of other constituents is considered.
3. The method is compared with the official assay procedure and with a nonaqueous titration method.

REFERENCES

- (1) "The National Formulary," 10th ed., AMERICAN PHARMACEUTICAL ASSOCIATION, Washington, D. C., 1955, p. 611.
- (2) Guenther, E., "The Essential Oils," Vol. I, D. Van Nostrand Co., New York, p. 291.
- (3) Hamarnah, S. K., Blake, M. I., and Miller, C. E., *THIS JOURNAL*, 45, 713 (1956).
- (4) "The United States Pharmacopeia," 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 1094.
- (5) Guenther, E., "The Essential Oils," Vol. III, D. Van Nostrand Company, New York, p. 755.
- (6) "The National Formulary," 7th ed., AMERICAN PHARMACEUTICAL ASSOCIATION, Washington, D. C., 1942, p. 306.

Sulfaethylthiadiazole III.*

Kinetics of Absorption, Distribution, and Excretion

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Experimental data are presented which describe blood concentrations during the early absorptive and post-absorptive periods following oral sulfaethylthiadiazole administration to humans. Data from urinary collections over forty-eight hours are also given. These and previously reported data are employed to illustrate and interpret the kinetics of sulfaethylthiadiazole absorption, distribution, and excretion. The pharmaceutical implications with regard to design and evaluation of data from dosage forms, and their regimens, are discussed.

PREVIOUS PAPERS (1, 2, 3) have reported the approximate first-order elimination rate of sulfaethylthiadiazole (SETD) as determined from human blood concentration and urinary excretion studies following intravenous and oral administration. Laboratory and clinical efficacy of SETD have also been reported (4, 5). This paper describes blood concentrations, observed during the early absorptive and post-absorptive phases; and urinary excretion data following oral SETD administration. These additional experimental data are correlated with data previously reported. The kinetics of SETD absorption, distribution, and excretion following oral and intravenous doses are interpreted in terms of these data, and some pharmaceutical implications related to problems of dosage form design are discussed.

EXPERIMENTAL

Eight healthy ambulatory adult human subjects were employed. Doses of SETD ranging from 1 to 4 Gm. were administered orally in 0.5-Gm. hard gelatin capsules just prior to breakfast, after control blood samples had been taken. Blood collections for sulfonamide concentration were made by venipuncture at 20, 30, 45, 65, 90, 120, 150, 180, and 240 minutes following administration.

In another study employing four subjects at the same oral doses, blood and urine collections were made. Following breakfast, the subjects were permitted to ingest foods and fluids as desired but were urged to consume a minimum of 300 cc. of water at each urine collection time. Collections of total urinary output were made at 0, 3, 6, 9, 12, 15, 24, and 48 hours. Blood concentration data were obtained from 0-10 hours and were interpreted previously (1).

Analytical Methods.—Concentrations of free as well as free plus conjugated (total) SETD in the

blood and urine were determined by a modification of the spectrophotometric macromethod of Frisk (6), as reported previously (1, 2).

RESULTS AND DISCUSSION

Blood Concentrations During First Four Hours.—The data for the eight subjects tested are given in Table I, and indicate rapid absorption. In almost all instances measurable blood concentrations were observed within twenty minutes; and with one exception, all subjects exhibited concentrations of free SETD equal to or in excess of 5 mg. % in sixty-five minutes. Peak blood concentrations generally occurred within one to three hours.

Urine Collection Studies.—The experimental urinary excretion data for four human subjects receiving SETD are presented in Table II. The close correspondence of free and total SETD excreted in the various time intervals corroborates results from intravenous administration studies that biotransformation is of a very low order (2). About 79-90% of the administered dose is excreted via the urinary tract in forty-eight hours. This contrasts with 83-100% excretion in forty-eight hours for subjects receiving 0.5-2.0 Gm. intravenous doses (2). After oral doses, an average of 85% of the drug was excreted in forty-eight hours; after intravenous doses, 91% was excreted in the same period. For these subjects one may assume for purpose of calculations that 93% of the total oral dose was absorbed and eventually excreted in the urine.

When the average total drug excreted during the three- to fifteen-hour interval is plotted by a differential method illustrated previously (2, 7), the biologic half-lives are of magnitudes observed for intravenous doses determined similarly. These plots are illustrated in Fig. 1. Biologic half-lives vary between six to eight hours. These and previous blood and urine concentration studies with SETD indicate that the biologic half-lives usually are within five to thirteen hours for the adult human subjects studied.

Distribution Volume.—It has been shown (2) for SETD that the apparent volume of distribution, V_b , determined from blood concentration data, may be estimated from the equation

$$V_b = W/C \quad (\text{Eq. 1})$$

where, W = mg. of total drug in the body at time t

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TABLE I.—BLOOD CONCENTRATION DATA DURING THE ABSORPTIVE AND EARLY POST-ABSORPTIVE PHASES FOR ADULT MALE SUBJECTS^a RECEIVING SETD AT SEVERAL DOSAGE LEVELS

Minutes	Dose (Gm.)	Subjects and Their Free (F) and Total (T) SETD Blood Concentration in mg. %															
		Bd				Bs ^b				Hn				Ro ^b			
		F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T
20	1.0	0.5	0.7	2.8	3.1	0.5	0.7	0.0	0.0	1.4	1.4	1.0	1.0	1.1	1.1	1.1	1.1
30		1.4	1.4	3.2	3.4	2.5	2.7	1.2	1.3	4.4	4.4	1.5	1.5	2.0	2.0	1.2	1.3
45		4.3	4.4	3.8	4.2	4.6	4.8	2.1	3.6	5.7	6.1	5.1	5.1	5.3	5.3	2.0	2.1
65		8.5	9.2	5.0	5.6	8.8	8.5	3.2	3.6	7.1	7.5	6.3	7.0	5.4	5.7	5.8	6.1
90		7.6	8.4	5.0	5.4	7.0	7.2	5.3	5.5	6.1	6.3	8.8	9.4	6.1	6.5	7.1	7.5
120		6.9	7.3	4.8	5.2	6.4	6.9	4.8	5.6	5.4	5.5	9.0	9.8	5.7	6.3	7.8	7.9
150		6.4	6.6	4.2	4.8	5.1	6.1	4.5	5.2	5.3	5.4	8.4	8.5	6.3	6.5	7.9	8.1
180		5.7	5.8	3.9	4.3	5.1	5.5	4.2	4.8	5.3	5.4	7.9	8.4	8.1	6.3	7.1	7.1
240		5.1	5.3	3.5	3.9	4.4	5.0	3.8	4.1	5.1	5.3	7.1	7.5	6.3	6.5	7.0	7.0
20	2.0	0.4	0.5	2.5	2.6	0.0	0.0	0.0	0.0								
30		4.0	4.1	7.2	7.9	0.4	0.3	0.0	0.0								
45		8.0	8.2	9.1	9.6	1.6	1.6	0.0	0.0								
65		11.6	11.8	9.8	10.4	10.2	10.2	5.0	5.0								
90		11.8	12.5	9.4	9.2	12.4	13.0	9.4	9.6								
120		10.2	11.0	9.0	9.1	11.2	11.6	10.2	10.2								
150		9.3	9.8	8.0	8.4	10.2	10.6	9.6	9.8								
180		9.0	9.7	7.6	7.6	9.4	9.6	8.8	9.3								
240		8.0	8.0	6.6	6.8	7.6	8.1	8.2	8.6								
20	3.0	0.9	1.0	0.8	0.9	1.4	1.5
30		1.4	1.5	1.0	1.1	5.3	5.4
45		2.1	3.0	6.7	7.1	7.0	8.1
65		7.7	7.9	9.2	10.8	8.5	9.2
90		7.9	8.0	12.5	13.0	9.2	9.3
120		8.5	9.7	14.0	14.3	11.3	11.8
150		13.1	13.1	15.2	15.6	12.5	13.1
180		12.5	13.0	15.8	16.4	13.1	14.7
240		12.0	12.5	11.8	13.1	13.1	13.0
20	4.0	2.7	2.8	9.1	9.7	2.0	2.6	0.0	0.0								
30		7.7	7.6	10.8	12.6	7.4	7.5	0.4	1.0								
45		10.9	10.6	12.0	12.7	19.7	11.2	2.2	3.4								
65		14.0	14.5	13.4	14.0	12.0	12.1	6.9	7.8								
90		18.2	18.2	13.9	14.6	17.5	19.2	11.8	12.6								
120		20.0	20.2	14.1	14.2	17.2	17.2	13.4	14.4								
150		18.6	19.8	13.5	13.5	15.1	15.6	13.5	14.4								
180		18.2	18.4	12.8	13.4	14.6	14.6	11.4	12.8								
240		16.4	15.9	11.5	12.0	13.2	13.8	10.6	11.8								

^a Subject ages and weights were as follows:

Subject	Age (yr.)	Weight (Kg.)	Subject	Age (yr.)	Weight (Kg.)
Bd	23	75	Mt	30	70
Bs	38	114	Ly	26	73
Hn	22	84	Bi	44	86
Ro	22	91	Ss	27	71

^b Blood concentration data at longer time intervals for oral and intravenous doses are reported for these subjects in previous papers (1, 2).

(and theoretically exclusive of any drug in the gastrointestinal tract and bladder); and $C =$ mg./cc. of total drug in the blood at time t , after establishment of diffusion equilibrium.

In light of this finding from intravenous studies on these same four subjects, it seemed desirable to determine if V_b had a similar value following oral dosage. As a corollary, it was of interest to establish further whether blood and urine data following oral doses would be correlated semi-quantitatively with results observed following intravenous doses. The pertinent experimental data and calculations are presented in Table III. In all calculations it was assumed that only 93% of the oral doses was absorbed. The average per cent distribution volume, V_b %, is observed to be about 20%, contrasting with 18% from intravenous studies. The correspondence is fair; however, the result from intravenous studies involves fewer assumptions and is therefore more reliable. Figure 2 is a plot of W vs. C from the data of Table III. Data for three subjects plot fairly satisfactorily as straight lines

approaching intersection with the origin. For the fourth subject the data did not show this consistency. The fair similarity between these plots and those observed following intravenous doses (2) supports the postulation that approximately a linear relationship exists between total SETD blood concentration and drug in the body. This relationship exists for blood concentrations up to at least 12–15 mg. % for these subjects.

Some Interpretations Applied to Experimental Data of a Single Subject.—The data of Tables I and II lend themselves to interesting theoretical interpretations and descriptive illustrations. Subject Bs has been studied intensively (1, 2) and has shown an SETD disappearance rate *via* blood and urine concentration studies quite typical of the average subject. It is convenient, therefore, to use data for this subject, following a 2-Gm. dose, to illustrate some of the interpretations pertaining to absorption and excretion that apply similarly to all the subjects.

TABLE II —URINARY EXCRETION DATA FOR HUMAN SUBJECTS RECEIVING ORAL SETD^a

Subject	Collection Time Interval (hr)	Urinary Volumes In cc and Drug Excreted in Mg for Intervals Indicated—											
		1 0 Gm Dose			2 0 Gm Dose			3 0 Gm Dose			6 0 Gm Dose		
		Urine vol (cc)	Free mg	Tot ^b mg	Urine vol (cc)	Free mg	Tot mg	Urine vol (cc)	Free mg	Tot mg	Urine vol (cc)	Free mg	Tot mg
Ro	0-3	160	141	160	250	340	369	680	571	578	135	713	770
Ro	3-6	190	122	125	175	287	312	200	480	540	163	737	782
Ro	6-9	152	94	100	175	266	294	213	320	330	166	478	523
Ro	9-12	160	67	77	260	166	174	865	277	294	133	261	279
Ro	12-15	235	80	96	165	109	112	330	165	188	96	175	187
Ro	15-24	335	77	87	495	193	193	710	284	298	467	411	453
Ro	24-48	1240	130	143	1760	220	229	1790	269	295	1470	338	397
Bs	0-3	300	174	180	115	340	385	385	647	712	118	699	773
Bs	3-6	1050	147	179	400	288	320	915	458	458	290	696	725
Bs	6-9	225	81	97	335	228	251	312	362	406	235	475	576
Bs	9-12	340	68	75	305	153	171	184	184	184	110	198	239
Bs	12-15	165	73	79	125	129	138	178	142	196	122	203	232
Bs	15-24	780	129	156	1020	255	291	750	270	315	625	450	463
Bs	24-48	1365	109	154	1300	189	233	1225	257	276	1443	375	375
Cw	0-3	72	109	119	190	365	376	220	757	836	435	726	740
Cw	3-6	84	148	166	660	325	351	455	505	569	570	536	604
Cw	6-9	125	150	153	100	182	190	120	221	252	150	420	435
Cw	9-12	130	81	85	95	114	126	78	225	250	248	441	477
Cw	12-15	510	92	92	160	186	192	125	175	194	340	187	204
Cw	15-24	2015	111	141	715	164	168	540	286	313	445	343	513
Cw	24-48	2580	129	142	1155	225	231	1350	277	297	2795	503	601
Ds	0-3	80	131	132	95	327	447	120	298	330	130	551	579
Ds	3-6	73	99	113	120	322	366	168	497	546	170	571	617
Ds	6-9	115	129	129	300	180	198	65	211	241	425	544	638
Ds	9-12	74	62	69	73	93	112	105	313	368	425	323	361
Ds	12-15	80	51	56	85	133	136	125	214	225	325	133	153
Ds	15-24	590	130	148	245	218	206	820	369	410	485	340	393
Ds	24-48	1035	176	186	1065	197	245	1220	329	378	1305	653	718

^a Data for the same subjects receiving intravenous SETD are reported in a previous paper (2).

^b Total drug represents free and conjugated drug expressed in mg equivalents of SETD

TABLE III —DATA CALCULATED FROM BLOOD CONCENTRATION AND URINARY EXCRETION STUDIES ON FOUR HUMAN SUBJECTS RECEIVING ORAL SETD

Subject	Height (in)	Wgt (Kg)	Age (yr)	Dose (Gm)	Total SETD blood concentration at		Total drug ^a in the body at 3 hrs (mg)	V _b (cc) from 3 hr data	Total SETD blood concentration at		Total drug ^a in the body at 6 hrs (mg)	V _b (cc) from 6-hr data	V _b (cc) Average from 3- and 6 hr data	V _b % (V _b /V _s)
					at 3 hrs (mg %)	%			at 6 hrs (mg %)	%				
Ro	78	91	22	1 0	4 3		770	19,700	3 5		645	20,300	21,000	23
				2 0	7 8		1491	21,000	6 3		1179	20,600		
				3 0	11 3		2212	21,500	8 5		1672	21,600		
				4 0	13.8	...			11 8					
Bs	72	114	38	1 0	4 1		750	16,100	2 9		571	17,300	19,000	17
				2 0	8 0		1475	16,200	5 5		1155	18,400		
				3 0	9 1		2078	20,100	6 6		1620	21,500		
				4 0	12 2		2947	21,200	9 2		2222	20,200		
Cw	74	91	22	1 0	6 1		811	14,600	4 6		645	15,400	17,000	19
				2 0	9 0		1484	18,100	7 3		1133	17,100		
				3 0	12 3		1954	17,500	8 8		1385	17,300		
				4 0	16 8		2980	19,500	13 6		2376	19,200		
Ds	71	81	25	1 0	7 4		798	13,300	6 1		685	13,900	18,000	22
				2 0	11 0		1413	15,900	9 0		1047	14,400		
				3 0										
				4 0	15 3		3141	25,400	14 3		2624	22,700		

^a Knowing the doses administered and assuming 93% absorption, the amounts of total SETD remaining in the body are determined by subtracting the cumulative amounts found in the urine at three and six hours respectively (Table II) from the amounts absorbed

The rate of SETD blood concentration change following an oral dose depends primarily on rate of absorption, sequestering and storage in specific tissues, rate and degree of biotransformation, rate of distribution between blood and other accessible body tissues, and rate of excretion. Previous data (1-3) have indicated that biotransformation, storage, and sequestering are minimal, and distribution proceeds at a rate similar to the absorption rate. Thus it is reasonable to assume that diffusion equilib-

rium is very nearly maintained during the absorption process, and that absorption and urinary excretion rates constitute the principal limiting rates which govern blood, body and urine content of a dose as a function of time. With these assumptions, and knowing that total SETD disappearance from the blood or body at therapeutic concentrations approximates a simple decreasing exponential rate after termination of absorption, equations similar in principle but different in form from those of Dominguez, *et al.* (8), and Teorell (9) may be applied to the experimental data. Use of these equations in conjunction with the experimental data for SETD permits a delineation of the interrelationships of absorption, distribution, body drug content, blood concentration, and urinary excretion as related to time.

Thus for the total SETD, let W be the amount which is present in body tissues at a given time. Some additional drug may or may not be in the gastrointestinal and urinary tracts. The rate at which W changes is equal to the difference between the rate of absorption dA/dt , and the rate of excretion dE/dt , i.e.

$$(dW/dt) = (dA/dt) - (dE/dt) \quad (\text{Eq. 2})$$

When $dA/dt = dE/dt$, W becomes constant and dW/dt therefore is zero, a consideration of practical interest in the design of oral sustained release dosage forms. One might predict from theory that such a dosage form could be designed to release a therapeutic dose for immediate absorption, and an amount each hour thereafter corresponding to the amount excreted per hour. The sustained release dose would be the product of total drug in the tissues corresponding to the desired therapeutic level, the specific velocity constant for drug elimination, and the number of hours such sustained absorption was desired (1-3, 10). In practice it is observed that other factors have a practical bearing on these calculations of dosage form design. These

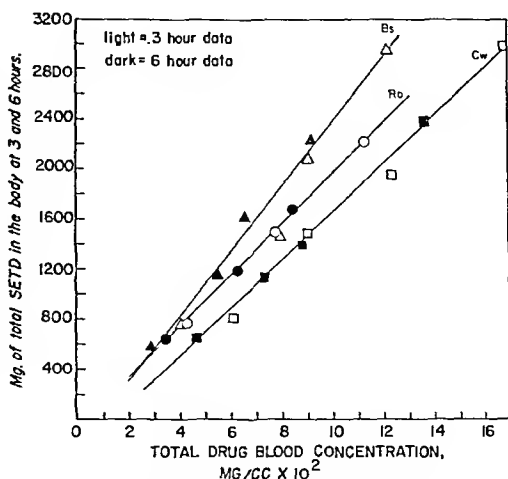


Fig. 2.—A plot of total SETD in the body vs. blood concentrations for subjects Bs, Ro, and Cw.

vary from one drug to another and for some drugs practically no oral sustained absorption can be effected by current technology.

Since V_b is assumed to be constant, and from Eq. 1, $W = V_b C$, one may substitute and transpose equivalent terms in Eq. 2 to obtain:

$$(dA/dt) = (V_b dC/dt) + (dE/dt) \quad (\text{Eq. 3})$$

The rate of total SETD urinary excretion has been shown to be approximately proportional to SETD concentration in the blood (2) and may be written:

$$dE/dt = k_b V_b C \quad (\text{Eq. 4})$$

where k_b is the specific velocity constant for drug elimination determined from blood concentration data. Substituting this equivalent value of dE/dt in Eq. 3

$$(dA/dt) = (V_b dC/dt) + (k_b V_b C) \quad (\text{Eq. 5})$$

After absorption ceases, the total SETD concentration in the blood falls, as previously reported (2), approximately according to the equation:

$$\log C = -(k_b t / 2.303) + \log C_0 \quad (\text{Eq. 6})$$

The values of k_b , V_b , and $V_b \%$ for subject Bs have been shown to be approximately 0.09, 20,000 cc., and 18% respectively (1, 2). Assuming 93% absorption of the oral doses, and employing the equations and constants enumerated above, the data in Tables I and II and those previously reported (1, 2) for subject Bs at a 2-Gm. dose, may be represented as shown in Fig. 3.

Curve A delineates the experimental blood concentration through ten hours (1) following oral administration. The remainder of the curve is extrapolated by use of Eq. 6. Curve B describes absorption rate of SETD from the data in Table I as interpreted with Eq. 5. It is evident that absorption is very rapid. The peak rate lies between 3000 and 4000 mg./hour, and is approximately 15-20 times faster than the peak excretion rate. The curve indicates that for this subject virtually all the absorbable drug is taken into the body within one to two hours. The area under the curve is a

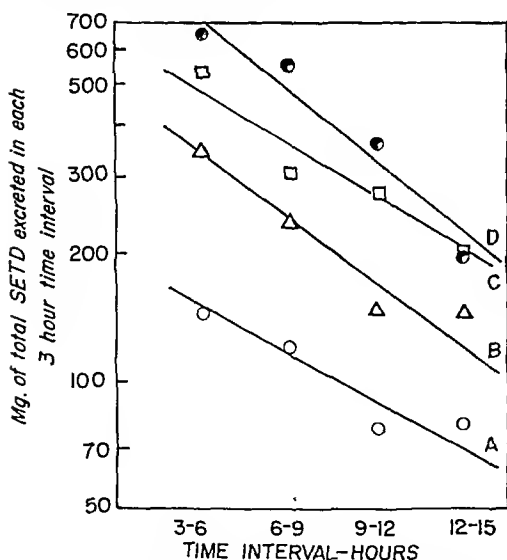


Fig. 1.—Plots of the average total SETD urinary excretions for 4 human subjects following oral doses of (A) 1.0, (B) 2.0, (C) 3.0, and (D) 4.0 Gm.

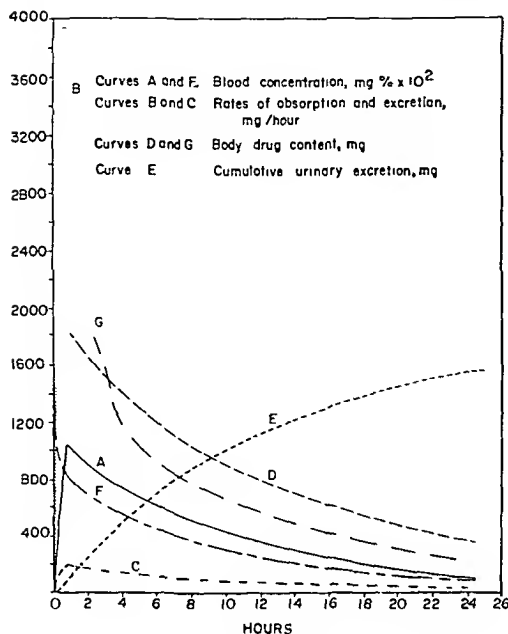


Fig. 3.—Total SETD data for subject Bs showing (A) blood concentration, (B) absorption rate, (C) excretion rate, (D) body content, (E) cumulative urinary collection curves after 2-Gm. oral dose, (F) blood concentration, and (G) body content curves after 2-Gm. intravenous dose.

measure of total drug absorbed (8); however, this quantity is determined indirectly with greater precision from urinary collection data.

Curve C describes SETD disappearance rate from the blood for an oral dose, calculated according to Eq. 4. Essentially the same curve is obtainable in from three–twenty-four hours using the urinary excretion data of Table II. The area under curve C is a direct measure of the total drug excreted in twenty-four hours; however, this quantity too is determined with greater precision from urinary collection data.

Curves D and E describe body content of the drug and drug collected cumulatively in the urine, respectively, with regard to time, from data of Table II. Curve F illustrates the experimental blood concentrations observed through eight hours following intravenous administration (2); the remainder of the curve was obtained by extrapolation employing Eq. 6. Curve G illustrates the experimental urinary excretion data corresponding to the blood concentration data of curve F. The urinary excretion rate is very rapid prior to attainment of diffusion equilibrium following the intravenous dose. Thereafter the shape of the curve corresponds to that observed in curve D for orally administered SETD.

Except for a slightly more rapid absorption than average, the composite data of Fig. 3 constitute a fairly accurate representation of the kinetics of SETD absorption, distribution, and excretion in the average healthy adult human subject of these studies. These data illustrate the following facts: drug disappearance rates, after absorption and diffusion equilibrium, are similar for orally or intravenously administered SETD, and from blood

concentration or urinary excretion data; sequestering or storage does not occur to any detectable extent; and absorption is efficient, with absorption rate being very rapid in comparison with excretion rate.

The data as portrayed in Fig. 3 have an application to dosage form design, determination of dosage regimens, and in the evaluation of blood and urine concentration data obtained from administration of conventional and sustained release dosage forms. For example, it is clinical practice to prescribe fixed doses of some chemotherapeutic agents at equally spaced times. Knowing the standard performance indices (1) it may be possible to calculate the "steady state blood concentrations" resulting from the oral administration of a given drug according to such a prescribed regimen, possibly minimizing time, effort, and cost in acquiring such data in pharmaceutical product development. Also, such data may be of value in the design of oral sustained release dosage forms because any change in drug release will necessarily influence absorption, excretion, and tissue concentrations of the drug. Theoretically, knowing the specific velocity constant for drug elimination from blood or urine drug concentration data coupled with a knowledge of *in vivo* drug absorption and distribution characteristics, should assist in the determination of the dose required to attain a certain tissue concentration or effect, and the absorption or release rate required to maintain it. These and other applications to SETD will be discussed further in future publications from these laboratories (10).

SUMMARY

1. Oral administration of SETD to humans results in rapid absorption of the drug, generally giving measurable blood concentrations within twenty minutes and peak therapeutic concentrations within one to three hours.

2. After absorption is complete and diffusion equilibrium is attained, orally administered SETD is excreted in a manner and at a rate comparable to an intravenous dose. Urinary excretion of SETD is rapid when contrasted with some other clinically available sulfonamides, an average of 85 per cent being excreted in forty-eight hours.

3. The per cent distribution volume, determined from blood concentration data after oral doses was 20 per cent, a value corresponding closely to that observed following intravenous doses.

4. These and previously reported data for this drug permit a delineation and interpretation of the kinetics of drug absorption, distribution, and excretion.

5. The importance of these data and interpretations to design problems of standard oral dosage forms and oral sustained release dosage forms and regimens is discussed.

REFERENCES

- (1) Swintosky, J. V., Robinson, M. J., Foltz, E. L., and Free, S. M., *THIS JOURNAL*, 46, 399 (1957).
- (2) Swintosky, J. V., Robinson, M. J., and Foltz, E. L., *ibid.*, 46, 403 (1957).
- (3) Foltz, E. L., Swintosky, J. V., and Robinson, M. J., *Federation Proc.*, 15 (1), 422 (1956).
- (4) Rhodes, R. E., Bard, R. C., and McCaughan, J. A., *Antibiotics & Chemotherapy*, 7, 175 (1957).
- (5) Farquhar, J. D., *J. Pediat.*, 50, 190 (1957).
- (6) Frisk, A. R., "Sulfanilamide Derivatives," *Acta Med. Scand.*, Supplement CXLII, Jan., 1943.
- (7) Swintosky, J. V., *Nature*, 179, 98 (1957).
- (8) Dominguez, R., and Pomerene, E., *Proc. Soc. Exptl. Biol. Med.*, 60, 173 (1945).
- (9) Teorell, T., *Arch. intern. pharmacodynamie*, 57, 205 (1937); *ibid.*, p. 226.
- (10) Swintosky, J. V., *et al.*, to be published.

Pharmacological Studies on Bis-(2-Methyl-2-Nitropropyl) Sulfite*

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Pharmacological studies on bis-(2-methyl-2-nitropropyl) sulfite indicate that this compound has sedative, hypotensive, and antispasmodic activity. The drug also possesses a negative chronotropic and negative inotropic action upon the frog heart. The results of a chronic toxicity study are also reported.

A PREVIOUS PUBLICATION (1) has reported the preparation of bis-(2-methyl-2-nitropropyl) sulfite, an aliphatic nitro compound which contains sulfur in an intermediate valence. This publication also reported the *in vivo* inhibition of this compound on Sarcoma 180 in mice, as well as the results of a toxicity study in week-old chicks. The apparent need for a more detailed pharmacological study of this type of compound, prior to synthesis of possible analogues, prompted the work to be reported herein.

EXPERIMENTAL

Bis-(2-methyl-2-nitropropyl) sulfite (hereafter designated BMNS) was synthesized and purified in our laboratory of pharmaceutical chemistry. Its identity was established from melting point data and solubility characteristics. The insoluble nature of BMNS required its preparation for injection in the form of a suspension in oil-in-water emulsions containing either cholesterol (2.5%) or lecithin (0.1%) as emulsifying agents. For the chronic toxicity study, an aqueous suspension containing 5% BMNS and 0.1% lecithin was utilized. An aqueous suspension of BMNS in 5% acacia was used for the smooth muscle studies. It was found that 1,4-dioxane, used as a solvent by Kessler, *et al.*

(1), was too toxic in the species employed in this study.

The pharmacological action of BMNS was determined in eleven unanesthetized rats and two unanesthetized cats. The drug was administered by either the oral, subcutaneous, or intraperitoneal routes. BMNS effects on the blood pressure and respiration were also determined in six rats, seven cats, and four dogs. The cats and dogs used in the blood pressure experiments were anesthetized intraperitoneally with pentobarbital sodium (35 mg./Kg.) and the rats with urethane (1 Gm./Kg., i. p.). Doses of 250 mg./Kg. of the sulfite were administered intraperitoneally, intramuscularly, or intravenously. The blood pressure responses were recorded from a cannulated carotid artery via a mercury manometer. Respiratory movements were recorded from a tambour connected to a tracheal cannula. The blood pressure effects of BMNS in rats injected intraperitoneally were recorded from a cannulated carotid artery through a special recording mercury manometer (2).

A 1:4000 aqueous suspension of BMNS was applied directly to the frog heart enclosed within a small cup. Contractions of the heart were recorded through a heart muscle lever upon a smoked kymograph drum. Six *Rana pipiens* frogs, weighing 50 to 60 Gm., were employed in this study. The effect of BMNS (1:4000 aqueous suspension) on electroshock threshold of the frog gastrocnemius sciatic preparation was also determined by conventional methods.

Five intestinal strips from three rabbits, two intestinal strips from a guinea pig, and three intestinal strips from two rats were utilized for studies on smooth muscle. The preparations were suspended in a 100-ml. muscle chamber containing either Tyrode's or Locke-Ringer's solution maintained at a temperature of 37.5-38.0°. Tyrode's solution was used for the guinea pig intestine, while the rabbit intestine was placed into Locke-Ringer's solution. The muscle responses were recorded by a long, writing lever on a smoked kymograph. The effects of a 1:40,000 aqueous suspension of BMNS were

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thus studied. The action of the sulfite on tracheal musculature was determined by the method described by Castillo and de Beer (3); tracheal chains from two guinea pigs were prepared for these experiments. The action of the sulfite (1:40,000 aqueous suspension) was also studied on the smooth muscle of the thoracic aorta of either guinea pig or bovine origin. The method of Furchgott and Bhadrakom (4) was employed.

Chronic toxicity studies of BMNS were made in both male and female albino rats of different ages by the daily intraperitoneal injection of 250 mg./Kg. for a period of twenty-eight days. At this time the animals were sacrificed by concussion and exsanguination and the internal organs examined grossly for evidence of pathologic processes.

RESULTS

Action in Unanesthetized Rats and Cats.—The drug was administered in a dosage of 250 mg./Kg. to rats and cats. Subcutaneous injection of the sulfite appeared to have little or no effect upon the rat. Oral administration of a similar dosage resulted only in production of diarrhea in approximately one and one-half to two hours. When given intraperitoneally, however, BMNS imparts to the animal a definite mild sedation occurring twenty minutes post-injection. The rat is easily aroused and is extremely adverse to handling, responding irritably to tactile stimulation. The cat fails to exhibit a similar sedative effect upon intraperitoneal injection of 250 mg./Kg. of the drug; on the contrary, this species appears to be somewhat more excited than the control animal. The degree of stimulation, however, is not as intense as that seen with small doses of morphine in the cat.

Action in Anesthetized Animals.—In normotensive rats doses of 250 mg./Kg. intraperitoneally lowered the blood pressure for periods up to three hours. The maximum average depressor response in all the normotensive animals utilized was approximately 40 mm. Hg with rapid onset and a duration of action in excess of two and one-half hours. Respiratory rate and amplitude were little affected throughout all of the blood pressure experiments. Normotensive cats and dogs exhibited a depressor response similar to that seen in rats (Fig. 1). The hypotension in the larger animals, however, was more persistent, lasting from three to five hours. The heart rate was decreased 5–8% below normal control levels; this effect was maximal one hour post-injection with gradual recovery to a normal rate. The depressor response in cats and dogs was evident only after intraperitoneal administration; intramuscular and intravenous injection appeared to be completely ineffective. Post-mortem examination of the animals revealed no evidence of unabsorbed BMNS in the peritoneal cavity. Similar examination of intramuscular sites, however, indicated that the sulfite was very poorly absorbed by this route.

Data obtained from rats and dogs with spontaneous, abnormally high, mean arterial pressure indicate that the drug is without effect as an antihypertensive agent. A depressor response could not be elicited if the pre-injection mean arterial pressure exceeded 150 mm. Hg.

Action on Frog Heart *in Situ*.—A 1:4000 aqueous suspension of BMNS produced a progressive slowing and decrease in amplitude of contraction of the frog heart *in vivo* (Fig. 2) with distinct evidence of partial A-V block of amplitude. There was no recovery from this effect. The effect was only par-

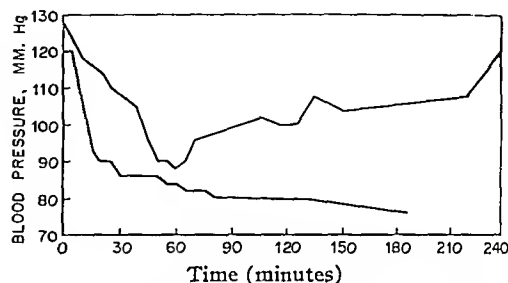


Fig. 1.—Upper curve—female cat, 4.2 Kg., pentobarbital sodium anesthesia, 35 mg./Kg., i. p. Note: Bis-2-methyl-2-nitropropyl sulfite, administered i. p. at zero time, causes a gradual, but marked fall of blood pressure with return toward normal occurring in four hours. The heart rate was decreased by approximately 6% at the end of one hour with a gradual recovery. The respiratory rate was little affected. Lower curve, female rat—0.315 Kg., urethane anesthesia, 1 Gm./Kg., i. p. Note: BMNS, administered i. p. exerts a hypotensive effect, which is rapid in onset and persists longer than two and one-half hours. At the end of this time the respiratory rate continued at the normal level of 72 per minute.

tially prevented by pre-treatment of the heart with atropine.

Action on Muscle-Nerve Preparation of Frog.—BMNS, (1:4000 in Ringer's solution) applied to the muscle-nerve preparation for fifteen minutes, has no effect on threshold stimuli required to produce

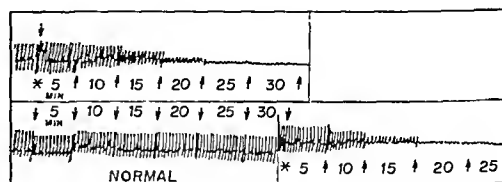


Fig. 2.—Effect of BMNS on the frog heart *in situ*. Administration of the drug is indicated by asterisk. Note the gradual slowing and decrease in amplitude of contraction. Results from two frogs were recorded simultaneously. The frog forming the lower tracing served as a control; the latter part of this tracing (beginning at asterisk) was formed by a confirmatory animal.

contraction of the gastrocnemius muscle either by stimulation of the sciatic nerve or the muscle directly.

Action on Isolated Smooth Muscle.—A 1:40,000 suspension of BMNS reduced the tone and amplitude of normally contracting intestinal smooth muscle strips. Spasms of the small intestine induced with 1:100,000 dilutions of methacholine were diminished by 1:40,000 aqueous suspension of BMNS. The muscle did not respond maximally again to methacholine until four doses of this drug were applied at intervals of ten minutes. The muscle was thoroughly washed two minutes before each subsequent addition of methacholine (Fig. 3). BMNS (1:40,000) also reduced the spasm of small intestine of guinea pigs, rats, and rabbits induced

there was some diffuse and pin-point hemorrhage of the lung in all the rats. There was much evidence of adhesion formation between the abdominal viscera and the serous layer of the peritoneal wall. Little unabsorbed drug occurred in the peritoneal cavity; however, deposition of the drug in the splenic tissue served as evidence of the activity of the phagocytic cells of the spleen and the minimal solubility of BMNS in body fluids.

DISCUSSION

Bis-(2-methyl-2-nitropropyl) sulfite, administered intraperitoneally, exerts a hypotensive action in anesthetized, normotensive rats, cats, and dogs with little effect on respiration and only minimal depression of cardiac rate. The compound appeared to be totally ineffective as an anti-hypertensive agent. Depression of the frog heart occurs when BMNS is applied directly; atropine is only partially effective in preventing the negative inotropic and chronotropic effects of the sulfite. Data from experiments on smooth muscle indicate that BMNS has a direct depressant action on smooth muscle organs, which may account for the depressor action in dogs, cats, and rats. Its slight depressant action on heart rate in cats and dogs probably does not account for the profound depressor action. It is not clear why the drug has a depressor action only when administered by the intraperitoneal route as opposed to other routes utilized in the study. It is a fact, however, that the major veins of the abdomen, because of their rather free mobility in the abdominal cavity, have somewhat greater ability to relax and to contract than do the veins of the remainder of the body, which are surrounded by solid structures. It may be postulated that BMNS, in intimate contact with these veins relaxes their musculature, resulting in a pooling of blood in these vessels and thus reducing peripheral mean arterial pressure. Further experimentation is necessary to confirm this hypothesis, as well as to investigate more fully the possibility of adrenergic blockade action.

It would appear that BMNS should have some merit as a gastrointestinal antispasmodic in view of its potency. Its extreme insolubility and apparent lack of absorption from the gastrointestinal tract, however, detract from its therapeutic efficacy.

REFERENCES

- (1) Kessler, W., Blake, M. I., and Miller, C. E., *This Journal*, 45, 570(1956).
- (2) "United States Pharmacopoeia," 15th Rev., Mack Publishing Co., Easton, Pa., 1955, p. 837.
- (3) Castillo, J. C., and de Beer, E. J., *J. Pharmacol. Exptl. Therap.*, 90, 104(1947).
- (4) Furchgott, R. F., and Bhadrakom, S., *ibid.*, 108, 129 (1953).

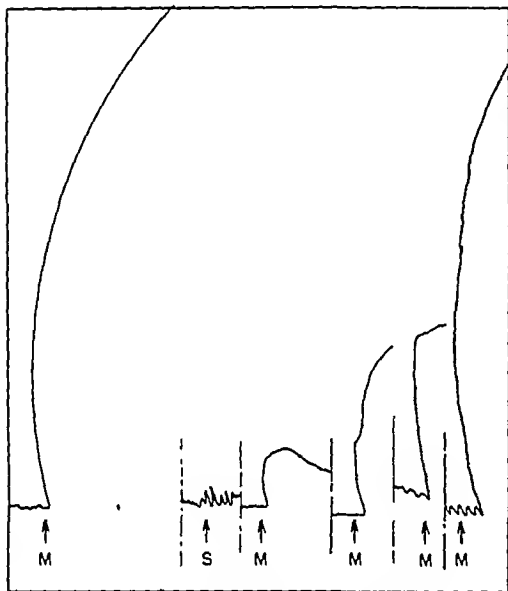


Fig. 3.—Action of BMNS on a strip of rabbit's isolated small intestine suspended in a 100-ml. bath of Locke's solution at 37.5°. Note: A 1:40,000 suspension of BMNS (S) blocked the stimulant effect of 1:100,000 solution of methacholine (M); also, four doses of methacholine ten minutes apart were required before the BMNS action was completely overcome. The muscle was washed two minutes before addition of drugs.

by varying concentration of barium chloride. The compound apparently was without effect on histamine-induced constriction of isolated tracheal smooth muscle (tracheal chain). BMNS (1:40,000) caused a complete relaxation of aortic smooth muscle placed in a state of moderate contraction by epinephrine (1:100,000,000 dilution).

Chronic Toxicity in Rats.—Chronic administration of BMNS to rats for four weeks appeared to induce no symptoms of toxicity which could be grossly observed in the intact animal. There was some exophthalmos observable up to one hour after injection. Red blood cell counts made at the termination of the study revealed that the drug depressed erythrocytic counts from 40–50% below normal control levels in more than one-half of the treated animals. Post-mortem examination indicated that

A Solubility Study of the Boric Acid-Water-Sorbitol System at Various Temperatures*

By JOHN J. SCIARRA,† JOHN AUTIAN,‡ and NOEL E. FOSS§

A study of the effect of various concentrations of sorbitol and temperature on the solubility and heat of solution of boric acid in water has been made. This system was studied at temperatures of 21°, 25°, 30°, 40°, 50°, and 60°. Concentrations of sorbitol ranging from 0 to 70 per cent by weight sorbitol in increments of 5 per cent were utilized in this study. The results of these experiments showed (1) the relationship between (1) solubility of boric acid and (2) temperature and concentration of sorbitol. A phase diagram of the boric acid-water-sorbitol system is given. The heat of solution for the various systems was calculated. The specific gravity of all solutions was determined by the pycnometric method.

BORIC ACID is soluble to the extent of about 5% by volume in water or 1:18 at 25° (1). This limited solubility of boric acid in aqueous solution has prompted many workers to investigate the effect of various solvents and other substances on the solubility of boric acid (2-5). A recent paper by Sciarra, Autian, and Foss reported that a 70% by weight sorbitol solution increased the solubility of boric acid from the above to 18.66% by weight or 1:3.45 at 25° (6). This represented a significant increase in the solubility of boric acid and seems worthy of further study; therefore, an investigation dealing with the solubility of boric acid in various concentrations of sorbitol and at various temperatures was made. From the data, the heat of solution of boric acid was evaluated and the application of the van't Hoff equation to this system was studied.

Boric acid reacts with sorbitol in solution to form a boric acid-sorbitol complex. Upon analysis of this solution, three components can be detected: boric acid, water, and sorbitol. This multicomponent system can be represented graphically by means of a phase diagram and

plotted on triangular graph paper. This diagram is useful for this system since the amount of sorbitol needed to dissolve a given amount of boric acid can readily be ascertained from the phase diagram.

Temperature is an important consideration when determining solubility. Generally, heat is absorbed when salts dissolve and, accordingly, they are more soluble at higher temperatures (7).

The solubility of a solid is a special case of an equilibrium constant (8). The relation between an equilibrium constant and the absolute temperature was developed by van't Hoff. An equilibrium constant enables one to predict how far a chemical reaction will go; it can be calculated for any temperature when it is known at two temperatures.

The mathematical relation is: $(d \ln S/dT) = (\Delta H/RT^2)$ and, when integrated,

$$\log S \approx (-\Delta H/2.303R \times 1/T + \text{constant}), \text{ or,} \\ \log S_2/S_1 = [\Delta H(T_2 - T_1)]/[2.303R(T_2T_1)]$$

where S_2 and S_1 are the solubilities, in moles per 1000 Gm. of solvent, at the absolute temperatures T_2 and T_1 ; R is the gas constant; and ΔH is the heat of solution in calories per degree per mole. In this integration ΔH is assumed to be constant, an assumption that is not entirely justified (8).

If a plot of the $\log S$ vs. $1/T$ yields a straight line, the slope of the line becomes equal to $-\Delta H/2.303R$. From this ΔH can readily be calculated.

EXPERIMENTAL

Solutions containing 0% sorbitol to 70% sorbitol by weight were prepared by diluting Sorbo® solution,¹ with the necessary amount of water. Each of these solutions was neutralized to phenolphthalein by adding a few drops of sodium hydroxide solution to produce a faint pink color. The solution was then placed in contact with an excess of finely divided crystalline boric acid, U. S. P., in a solubility tube. The solubility tube was then placed in the thermostat and stirred constantly for twenty-four hours. After stopping the stirring motor and allowing the undissolved particles to settle to the bottom, 25-ml. portions of the clear supernatant liquid were removed, placed into tared beakers, and quickly

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Adapted from the manuscript submitted by John J. Sciarra which received honorable mention in the 1957 Lunsford Richardson Pharmacy Awards competition.

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¹ Seventy per cent sorbitol, Atlas Powder Co., Wilmington, Del.

weighed. The amount of boric acid in these solutions was determined by assaying the solution according to the U. S. P. assay for boric acid (1).

At the same time, a 25-ml. Gay-Lussac specific gravity bottle was filled with the saturated solution of boric acid in the sorbitol solvent and the specific gravity determined by the pycnometric method (9). The specific gravity of the sorbitol solvent alone was also determined.

The solubility of boric acid and specific gravity of each of the above solutions were determined at 21°, 25°, 30°, 40°, 50°, and at 60° \pm 0.1°.

RESULTS

The results of the above experiments were treated in several different ways in order to best show the relationship existing among the various solutions.

Tables I and II give the specific gravity of sorbitol solutions and of saturated boric acid solutions in sorbitol at various temperatures as determined by the pycnometric method. Specific gravity of a solution of boric acid in sorbitol solution *vs.* per cent weight sorbitol at 21° and 60° was plotted and is shown in Fig. 1.

Table III gives the solubility of boric acid in sorbitol solutions at various temperatures. Figure 2 shows the solubility of boric acid in sorbitol solutions at various temperatures while Fig. 3 shows the solubility of boric acid at various concentrations of sorbitol.

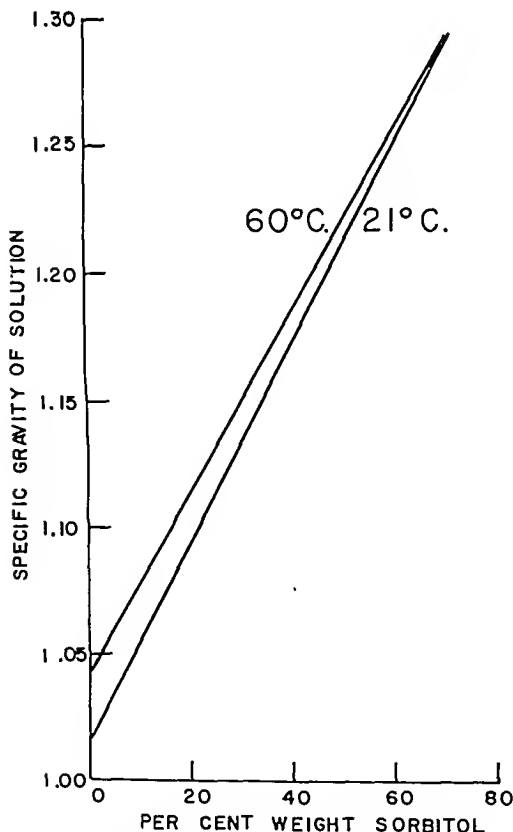


Fig. 1.—Isotherm curves showing specific gravity of saturated solution of boric acid in sorbitol.

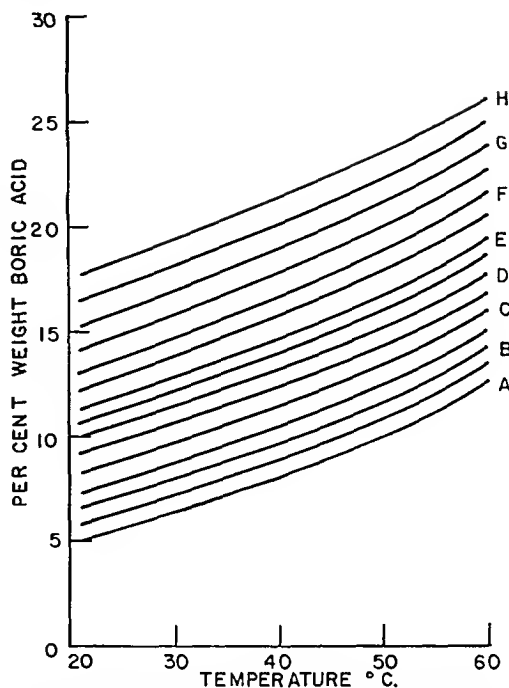


Fig. 2.—Effect of temperature upon the solubility of boric acid in sorbitol solution. A—zero, B—10, C—20, D—30, E—40, F—50, G—60, H—70, per cent weight sorbitol.

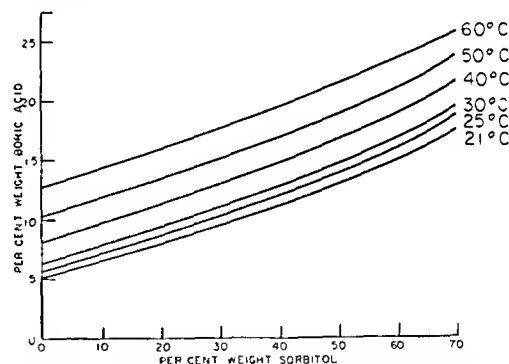


Fig. 3.—Isotherm curves showing solubility of boric acid in sorbitol solution.

The composition of various solutions consisting of boric acid, sorbitol and water is given in Table IV. The results at 25°, 40°, and 60° were plotted on triangular graph paper and are shown in Fig. 4.

Figure 5 shows the relationship between the log of boric acid solubility in moles per 1000 Gm. of solvent and reciprocal of absolute temperature. From this graph the slope was determined from which ΔH was calculated. Then, by substituting these values of ΔH in van't Hoff's equation the solubility of boric acid was calculated at 35° and compared with the value obtained from Fig. 2. These results are shown in Table V.

DISCUSSION

The specific gravity of sorbitol solutions was found to increase with an increase in concentration

TABLE I.—SPECIFIC GRAVITY OF SORBITOL SOLUTIONS AT VARIOUS TEMPERATURES

Concn of Sorbitol, % w/w	Specific Gravity					
	21°	25°	30°	40°	50°	60°
0	1.000	1.000	1.000	1.000	1.000	1.000
5	1.019	1.018	1.018	1.017	1.017	1.017
10	1.037	1.034	1.036	1.036	1.036	1.035
15	1.056	1.055	1.054	1.054	1.053	1.054
20	1.075	1.074	1.075	1.074	1.074	1.073
25	1.096	1.095	1.095	1.094	1.093	1.093
30	1.118	1.116	1.115	1.115	1.115	1.114
35	1.137	1.137	1.135	1.134	1.133	1.133
40	1.151	1.150	1.150	1.148	1.147	1.157
45	1.181	1.178	1.179	1.177	1.176	1.179
50	1.202	1.200	1.200	1.198	1.195	1.200
55	1.227	1.225	1.226	1.224	1.224	1.226
60	1.250	1.249	1.248	1.247	1.244	1.251
65	1.275	1.273	1.274	1.272	1.271	1.279
70	1.298	1.298	1.289	1.297	1.295	1.291

TABLE II.—SPECIFIC GRAVITY OF SATURATED SOLUTION OF BORIC ACID IN SORBITOL SOLUTION AT VARIOUS TEMPERATURES

Concn of Sorbitol, % w/w	Specific Gravity					
	21°	25°	30°	40°	50°	60°
0	1.018	1.020	1.023	1.029	1.036	1.046
5	1.036	1.038	1.041	1.047	1.053	1.061
10	1.056	1.056	1.059	1.064	1.071	1.079
15	1.074	1.075	1.077	1.082	1.084	1.096
20	1.095	1.095	1.096	1.101	1.108	1.114
25	1.113	1.114	1.116	1.120	1.124	1.131
30	1.134	1.134	1.140	1.140	1.145	1.150
35	1.152	1.152	1.154	1.157	1.163	1.167
40	1.165	1.164	1.166	1.170	1.171	1.187
45	1.190	1.190	1.192	1.194	1.197	1.206
50	1.211	1.210	1.210	1.210	1.214	1.221
55	1.231	1.231	1.232	1.234	1.235	1.240
60	1.253	1.252	1.252	1.254	1.257	1.258
65	1.274	1.272	1.272	1.271	1.273	1.272
70	1.294	1.292	1.293	1.291	1.290	1.294

TABLE III.—SOLUBILITY OF BORIC ACID IN SORBITOL SOLUTION AT VARIOUS TEMPERATURES

Concn of Sorbitol, % w/w	Solubility											
	21°		25°		30°		40°		50°		60°	
	A ^a	B ^b	A	B	A	B	A	B	A	B	A	B
0	18.88	5.03	16.98	5.57	14.73	6.36	11.36	8.09	9.03	9.98	6.91	12.65
5	16.13	5.74	14.60	6.31	12.65	7.21	10.01	8.95	8.01	10.95	6.33	13.46
10	13.45	6.70	12.68	7.09	11.12	7.99	8.91	9.78	7.25	11.76	5.82	14.25
15	11.98	7.33	11.10	7.87	9.87	8.76	7.97	10.64	6.45	12.84	5.38	15.00
20	10.19	8.37	9.75	8.72	8.74	9.63	7.13	11.55	5.93	13.56	4.90	16.00
25	9.14	9.09	8.73	9.47	7.83	10.45	6.42	12.46	5.43	14.43	4.51	16.88
30	8.17	9.88	7.71	10.42	7.04	11.31	5.93	13.14	4.98	15.26	4.15	17.80
35	7.36	10.68	7.00	11.18	6.40	12.11	5.41	14.02	4.53	16.33	3.87	18.60
40	6.88	11.22	6.62	11.63	6.01	12.64	5.12	14.53	4.36	16.69	3.56	19.57
45	6.19	12.03	5.72	12.92	5.28	13.86	4.52	15.83	3.88	17.97	3.28	20.52
50	5.63	12.87	5.20	13.80	4.84	14.72	4.17	16.67	3.59	18.94	3.00	21.76
55	5.01	13.99	4.64	14.98	4.32	15.89	3.76	17.87	3.26	20.02	2.80	22.60
60	4.44	15.28	4.15	16.17	3.91	17.02	3.44	18.91	2.97	21.31	2.45	24.63
65	3.98	16.45	3.75	17.31	3.50	18.32	3.10	20.27	2.75	22.30	2.41	24.48
70	3.66	17.43	3.37	18.66	3.19	19.47	2.41	24.20	2.48	23.74	2.25	25.65

^a A—Solubility of boric acid expressed as ml. of solvent/Gm. of solute.
^b B—Solubility of boric acid expressed as per cent by weight.

of sorbitol and decrease with an increase in temperature. The specific gravity of the various solutions varied from 1.000 for pure water (0% sorbitol) to 1.291 for a 70% solution of sorbitol at 60°. As shown in Fig. 1, the relationship existing between specific gravity of a saturated solution of boric acid in sorbitol solution and concentration of sorbitol is a linear function. While the specific gravity of sorbitol solution was found to decrease with an increase in temperature, the reverse is true for the saturated solution of boric acid in sorbitol solution. The specific gravity of saturated solutions of boric acid in sorbitol solution was found to increase with an increase in temperature. This is attributed to the increase in concentration of boric acid brought about by the increase in temperature.

TABLE IV.—COMPOSITION OF BORIC ACID-WATER-SORBITOL SYSTEM AT VARIOUS TEMPERATURES

			Composition					
Boric Acid, %	Sorbitol, %	Water, %	Boric Acid, %	Sorbitol, %	Water, %	Boric Acid, %	Sorbitol, %	Water, %
21°			25°			30°		
5.03	0.00	94.97	5.57	0.00	94.43	6.36	0.00	93.64
5.74	4.76	89.50	6.31	4.68	89.01	7.21	4.64	88.15
6.70	9.33	83.97	7.09	9.29	83.62	7.99	9.20	82.81
7.33	13.90	78.77	7.87	13.82	78.31	8.76	13.69	77.55
8.37	18.33	73.30	8.72	18.26	73.02	9.63	18.07	72.30
9.09	22.73	68.18	9.47	22.63	67.90	10.45	22.39	67.19
9.88	27.04	63.08	10.42	26.87	62.71	11.31	26.61	62.08
10.68	31.26	58.06	11.18	31.09	57.73	12.11	30.76	57.13
11.22	35.51	53.27	11.63	35.47	52.90	12.64	34.94	52.42
12.03	39.59	48.38	12.92	39.19	47.89	13.86	38.76	47.38
12.87	43.57	43.56	13.80	43.10	43.10	14.72	42.64	42.64
13.99	47.31	38.70	14.98	46.76	38.26	15.89	46.26	37.85
15.28	50.83	33.99	16.17	50.30	33.53	17.02	49.79	33.19
16.45	54.31	29.24	17.31	53.75	28.94	18.32	53.09	28.59
17.43	57.80	24.77	18.66	56.96	24.38	19.47	56.37	24.16
40°			50°			60°		
8.09	0.00	91.91	9.98	0.00	90.02	12.65	0.00	87.35
8.95	4.55	86.50	10.95	4.45	84.60	13.46	4.33	82.21
9.78	9.02	81.20	11.76	8.82	79.42	14.25	8.58	77.17
10.64	13.40	75.96	12.84	13.07	74.09	15.00	12.75	72.75
11.55	17.69	70.76	13.56	17.29	65.15	16.00	16.80	67.20
12.46	21.89	65.65	14.43	21.39	64.18	16.88	20.78	62.34
13.14	26.06	60.80	15.26	25.42	59.32	17.80	24.66	57.54
14.02	30.07	55.91	16.33	29.28	54.39	18.60	28.84	52.56
14.53	34.19	51.28	16.69	33.34	49.97	19.57	32.57	47.86
15.83	37.88	46.29	17.97	36.91	45.12	20.52	35.77	43.71
16.67	41.67	41.66	18.94	40.53	40.53	21.76	39.13	39.11
17.87	45.17	36.96	20.02	43.99	35.99	22.60	41.47	35.93
18.91	48.65	32.44	21.31	47.21	31.48	24.63	45.22	30.15
20.27	51.82	27.91	22.30	50.51	27.19	24.48	49.09	26.43
24.20	52.96	22.84	23.74	53.38	22.88	25.65	52.05	22.30

The solubility of boric acid in sorbitol solutions was found to increase with an increase in temperature and concentration of sorbitol. The solubility of boric acid varied from 5.03% in water at 21° to 25.7% in 70% sorbitol solution at 60°. Figures 2 and 3 can be used to determine the solubility of boric acid in sorbitol solutions of 0 to 70% by weight at temperatures ranging from 21° to 60°. This relationship is important when one considers problems of formulation, stability, and storage where slight changes in temperature may seriously affect the solubility.

The composition of each of the solutions, in per cent by weight of sorbitol, boric acid, and water, was plotted on triangular graph paper. The maximum concentration of boric acid was at 60° and found to be 25.7% at a concentration of 52.1% sorbitol and 22.2% water. At higher concentrations one or more of the components precipitated. A phase diagram is very useful in determining concentrations when one or two components are fixed at a definite value. For example, at 25°, in order to dissolve 8% boric acid, 14% of sorbitol and 78% of water is required. This solution will be saturated with boric acid at this temperature. The area above the curves in Fig. 4 represents concentrations of the three components which will yield a clear solution. One or more components will precipitate in the area beneath the curves. Theoretically, a maximum solubility is reached at about 50 to 60% sorbitol (depending upon temperature) and it is believed that higher concentrations of sorbitol will actually result

in a lesser solubility of boric acid. Since both the boric acid and sorbitol will be competing for the lesser amount of water present, the sorbitol, having the greater solubility, will dissolve at the expense of the boric acid. This phenomenon is further shown on the phase diagram, since the curves must approach the point representing 100% sorbitol, 0% water, and 0% boric acid, which is located at the apex of the triangle. As can be seen from the curves the increase in temperature lowers the curves, increasing the area above the curve resulting in a

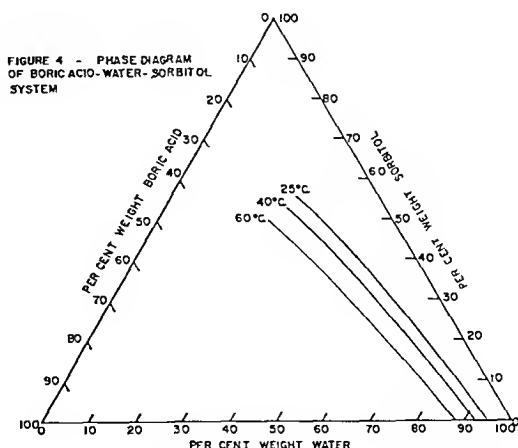


Fig. 4—Phase diagram of boric acid-water-sorbitol system.

greater area of solubility for boric acid in the sorbitol solvent.

Figure 5 shows the log of solubility *vs* reciprocal of absolute temperature. This plot yielded a straight line from which the slope was determined. Calcula-

TABLE V—COMPARISON OF CALCULATED SOLUBILITY WITH EXPERIMENTALLY DETERMINED SOLUBILITY OF BORIC ACID

A	B	C	D	E
0	5 030	1 223	1 257	1 236
5	4 580	1 405	1 399	1 406
10	4 260	1 560	1 558	1 551
15	4 030	1 755	1 723	1 699
20	3 840	1 895	1 907	1 897
25	3 470	2 082	2 058	2 081
30	3 420	2 247	2 269	2 268
35	3 200	2 475	2 426	2 416
40	3 200	2 541	2 537	2 556
45	3 200	2 779	2 860	2 798
50	3 050	2 997	3 060	2 989
55	2 900	3 249	3 340	3 254
60	2 750	3 556	3 576	3 550
65	2 470	3 848	3 875	3 792
70	2 410	4 193	4 245	4 144

A—Concentration of sorbitol, per cent by weight B— ΔH calculated from slope calories/mole/degree C—Calculated solubility at 35° when $T_1 = 323^\circ \text{K}$, moles/1000 Gm solvent D—Calculated solubility at 35° when $T_1 = 298^\circ \text{K}$, moles/1000 Gm solvent E—Solubility determined experimentally from Fig 2 moles/1000 Gm solvent

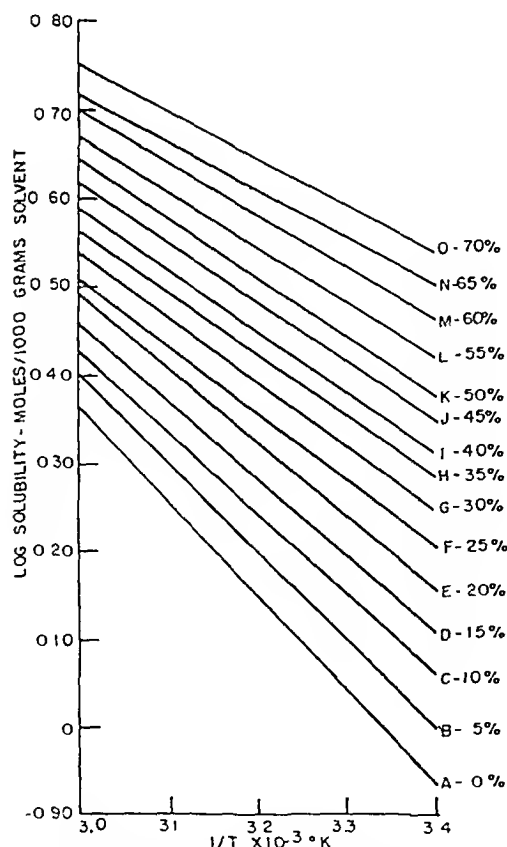


Fig. 5—Plot of log solubility of boric acid *vs* reciprocal of absolute temperature

lation of ΔH for each of the solvents ranging from water (0% sorbitol) to 70% sorbitol, shows that ΔH decreases with an increase in concentration of sorbitol. This change indicates that less heat is involved when a mole of boric acid dissolves in 70% sorbitol as compared to dissolving in water or in a lesser concentration of sorbitol. The application of van't Hoff's equation to this system was tested and found to give acceptable results as shown in Table V. Utilizing this equation, the solubility of boric acid can be calculated at any temperature. This constant variant is important in pharmaceutical formulations since a knowledge of solubility enters into many problems.

SUMMARY AND CONCLUSIONS

1. A solubility study of the boric acid-water-sorbitol system has been made at various temperatures.

2. The specific gravity of sorbitol solutions was determined by the pycnometric method and found to increase with an increase in concentration of sorbitol, and decrease with an increase in temperature. While the specific gravity of sorbitol solution was found to decrease with an increase in temperature, the reverse was found to be true for the saturated solution of boric acid in sorbitol solution. This was due to the increased solubility of boric acid at elevated temperatures.

3. The solubility of boric acid in sorbitol solution was determined at various temperatures and concentrations of sorbitol and found to increase with an increase in temperature and concentration of sorbitol.

4. A phase diagram of the boric acid-water-sorbitol system was determined at various temperatures. The heat of solution, ΔH , for the various systems was evaluated by plotting log of solubility of boric acid *versus* reciprocal of absolute temperature. It was found that ΔH decreased with an increase in concentration of sorbitol. The application of van't Hoff's equation to this system was tested and found to give acceptable results.

REFERENCES

- (1) "Pharmacopeia of the United States of America," 15th Rev., Mack Printing Company, Easton, Pa., 1955, p 103.
- (2) Holm, *Pharm Weekblad*, 58, 860(1921); through *Chem Abstr*, 15, 2962(1921).
- (3) Miner, C S, and Dalton, N. N., "Glycerol," Reinhold Publishing Co., New York, 1953.
- (4) Kendall, J, and Andrews, J. C., *J. Am Chem Soc*, 43, 1845(1921).
- (5) Ryss, I G., Slutskaya, M. M., and Vitukhnovskaya, B. S., *J. App Chem U S S R*, 25, 157(1952); through *Chem Abstr*, 48, 8014(1954).
- (6) Searra, J. J., Autian, J., and Foss, N. E., *THIS JOURNAL*, 13, 366(1957).
- (7) Daniels, F., and Alberty, R., "Physical Chemistry," John Wiley & Sons, Inc., New York, 1955, p 277.
- (8) Daniels, F., Mathews, J. H., and Williams, J. W., *et al*, "Experimental Physical Chemistry," 4th ed., McGraw-Hill Book Co., Inc., New York, 1949, p. 125.
- (9) *Ibid*, pp. 101, 427.

The Distribution and Fate of Iodine in Iodopropylidene Glycerol in the Rat*

By GEORGE F. HOFFNAGLE† and ARTHUR OSOL

The distribution and fate of iodine in iodopropylidene glycerol in the rat were studied by use of the compound tagged with iodine¹³¹, and comparisons were made with a similar study of tagged potassium iodide, both substances being given in a single dose of a magnitude equivalent to the therapeutic dose of potassium iodide in humans. Iodopropylidene glycerol was found to provide more prolonged though lower blood iodide levels and higher thyroidal iodine levels than an equivalent dose of potassium iodide.

THE HISTORY of systemic iodine therapy is long and confused, and the application of such therapy has traversed the gamut of diseases. In the past half century, various clinical and laboratory studies have established the value, in greater or less degree, of iodine therapy in many disease entities. The practical problems incident to systemic iodine therapy have been chiefly those of gastric irritation caused by iodides and elemental iodine, and the onset of iodism attending continued high-level therapy.

One compound which appears to produce desirable clinical benefits without causing gastric distress or iodism is iodopropylidene glycerol (1-5), hereafter called IPG.¹ The apparent advantages of IPG prompted this investigation of its biological distribution and utilization.

The lack of gastric distress is undoubtedly attributable both to the absence of iodide ion and free iodine in the preparations of the compound and, by virtue of the stability of the compound in acid media, to the lack of release of these in the stomach.

Biological work done elsewhere indicates the compound to be devoid of demonstrable chronic toxicity at reasonable levels, and to have an LD₅₀ of approximately 700 mg. per Kg. of body weight when injected intraperitoneally into mice; its intestinal absorption is equal to, or exceeds, that of Lugol's solution; and it produces the same levels of protein-bound iodine in blood as do inorganic iodides (6, 7). Its efficacy and efficiency for iodine therapy have been established.

EXPERIMENTAL

In order to determine the distribution of a single therapeutic dose of iodine, the approximate equivalent for rats of the amount of iodine represented in the average human dose of potassium iodide (300 mg.) was used, this amounting to 1 mg. of iodine per rat, given as 1.3 mg. of potassium iodide or 2 mg. of IPG. The potassium iodide, tagged with 100 microcuries of I¹³¹, was given in 1 ml. of water. The IPG, tagged with variable radioactivity depending on the tagging achieved, was given in 1 ml. of 60% v/v glycerin solution orally, or 20% v/v glycerin solution intravenously. No evidence of damage to thyroid tissue by radiation was observed either macroscopically or by gland weights in any experiment.

Young male Wistar rats from 250 to 300 Gm. in weight were brought to iodine equilibrium by feeding solely on Purina Dog Chow for two weeks prior to the experiments. Immediately after giving the dose orally using a feeding needle, the rats were placed in metabolism cages mounted on shallow funnels. The funnels were fitted with paper toweling with the center hole open, the toweling being treated with sodium hydroxide solution. The feces could thus be collected in sodium hydroxide solution under the funnel, while the alkali on the paper achieved immediate absorption of all urine and prevented loss of iodine by decomposition to elemental form.

Five rats were used for each time period in each experiment. The rats were sacrificed by ether anesthesia at the specified times and immediately dissected. The tissues were studied by the following procedures. The thyroids were digested individually by the Blau procedure (8), involving an eight-hour period of refluxing in 8% barium hydroxide solution, with subsequent separation into a thyroxine fraction and a non-thyroxine fraction by *n*-butanol extraction of the digested solution containing added sodium hydroxide.

The lungs, liver, spleen, kidneys, stomach, small intestines and contents, large intestine and contents, skeletal muscle, brain, fat (from between the shoulder blades, being careful to use only the white fat), knee joint (with the bone on each side cropped close and the muscle carefully removed), and eye—both whole and lens only, were pooled to obtain one sample of each type of tissue from each five rats, and were digested at boiling temperature in 4*N* sodium hydroxide solution. The whole blood was counted individually, directly as a 1:1 dilution in anticoagulant citrate solution U. S. P., since Owen and

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Abstracted in part from a dissertation submitted by George F. Hoffnagle to the Graduate School of the Philadelphia College of Pharmacy and Science in partial fulfillment of the requirements for the degree of Doctor of Science.

This investigation was supported in part by a grant from Henry K. Wampole & Co., Inc., Philadelphia, Pa.

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‡ Preparations of IPG used in this study were supplied by Henry K. Wampole & Co., Inc., Philadelphia, Pa.

Power (9) have shown that the blood iodide distribution is 0.565 times as high in the cells as it is in the plasma. The bone was digested in hot sulfuric acid-chromic acid solution, thereby converting the iodine to iodate. All digested solutions were brought to definite volumes in volumetric flasks, and 2-ml. aliquots were counted in a lead-shielded counting chamber using an end-window, organic-quenched, Geiger-Mueller counter tube and a Tracerlab Superscaler. All counts were corrected for dead time, background, to RaDEF standard, for decay, and for dilution. The counts per minute obtained for the *n*-butanol samples must be reduced by 15% for comparison with the aqueous samples, due to the difference in self-absorption in the sample caused by the change of solvents. Calculations were made in terms of counts per total tissue and/or counts per Gm. of tissue, as indicated. These were then converted to per cent of total dose.

No attempt was made to do more than follow the distribution of the iodine in this series. Preliminary experiments indicated that the iodine in IPG is completely converted to inorganic iodide by both of the alkaline digestive procedures used in the analysis.

The results represent the average for 10 rats at each time interval where standard deviation is given, or the average for five rats where standard deviation is not given, and for five rats for all values at the seventy-two-hour period. The important results, given in Table I, are on a wet tissue basis. The values for kidneys, liver, lungs, skeletal muscle, spleen, joint, bone, fat, eye, lens, and brain were proportionately lower than, and followed closely, the blood levels.

Chromatographic Study of Thyroid, Blood, and Urine 24 Hours after Administration of IPG or Potassium Iodide.—Rats administered the equivalent of 1 mg. of iodine, as tagged IPG or potassium iodide, given orally or intravenously, were sacrificed at twenty-four hours and blood, thyroid, and urine samples were obtained. The blood was obtained by excising the heart, and immediately diluted with an equal volume of anticoagulant citrate solution; the radioactivity of a 2-ml. sample was determined, and the remainder centrifuged to obtain a plasma sample. The excised gland was immediately weighed, extracted with *n*-butanol by a modification of the Gross and Leblond (10) method, using a revolving Teflon pestle in a Pyrex tube mortar, 0.1 ml. of water added to accomplish the contusion, followed by 1 ml. of *n*-butanol to complete the disintegration and to extract the iodinated contents. *n*-Butanol was shown to be a good solvent for IPG as well as all normal thyroid iodine compounds (10). This process yielded 1 ml. of solution, from which the solid sediment was removed by centrifugation; the radioactivity of this solution was measured.

The total thyroid extract, 0.1 ml. of the 1:1 diluted plasma, and 0.1 ml. of the twenty-four-hour urine, along with radioactive sodium iodide and IPG standards, were placed on Whatman No. 1 filter paper sheets by pipetting 0.006-ml. portions on the origin spot at a time, drying each application with the aid of mild heat and a current of air. Chromatography was performed by an ascending technique, using *n*-butanol saturated with pH 7.2

phosphate buffer, for a period of approximately twelve hours. In conjunction with this phase of the study it was demonstrated: that iodide ion is trapped by plasma and exhibits little or no chromatographic movement, regardless of whether the two are brought together *in vivo* or *in vitro*; that IPG, placed on the paper first, with normal plasma afterward placed on it, migrated as expected according to the IPG standard; that IPG superimposed by urine also migrated as expected. Chromatograms were air-dried for approximately one-half hour, then scanned for radioactivity on a scanning table (54). A complete set of the chromatograms of one rat in each dose category were autoradiographed.

The chromatograms indicated that: no IPG was present in blood, urine, or thyroid at twenty-four hours; iodide ion was always present in all three tissues at twenty-four hours, regardless of whether IPG or potassium iodide was given or by what route; occasional rats yielded sufficient radioactive thyroxin-like material to be detectable, though of a very low order. This last is in contradistinction to the results of Gross and Leblond (10), who used carrier-free radioiodide which was taken up and converted by thyroid with the result that much higher radioactivity levels were obtained.

Studies on Initial Distribution of Iodopropylidene Glycerol in the Rat.—In this phase of the study selected solid tissues of rats receiving tagged compounds were homogenized in a Waring Blendor with 0.1% potassium iodide solution; and the radioactivity of aliquots of these homogenates, as well as of blood samples, was measured. Then chloroform extracts were prepared by thorough agitation of the homogenates and of the whole blood with measured volumes of chloroform. Phase separation was effected by centrifugation. Aliquot portions of the chloroform extracts were counted for an estimate of the IPG portion of the total activity previously measured. The IPG was again the I^{131} -tagged material prepared as previously described. Measurements of the radioactivity were made on 2-ml. samples enclosed in a 2-inch lead shield, using an end-window, organic-quenched, Geiger-Mueller counter tube and a Tracerlab Superscaler. The solubility ratio of IPG in chloroform-water systems and the variation of self-absorption in chloroform solutions from the self-absorption in water (19% greater self-absorption in chloroform) were taken into account.

The rats were anesthetized by administering 35 mg./Kg. of pentobarbital sodium intraperitoneally, occasionally supplemented at longer time intervals with ether anesthesia at the end of the experimental period in order to terminate the individual experiments at the specified time. Oral and intravenous routes of administration were compared in order to throw more light on the question of absorption from the gastrointestinal tract.

Sufficient paper chromatograms (11) were run on the chloroform extracts to show that activities reported for these chloroform extracts are due to IPG as such. *n*-Butanol saturated with pH 7.2 phosphate buffer solution was used as the solvent. Results of these studies are shown in Figs. 1 and 2, and Table II.

TABLE I.—RESULTS OF I^{131} DISTRIBUTION STUDIES ON POTASSIUM IODIDE AND IPG, AFTER SINGLE ORAL DOSE CONTAINING 1 MG. OF IODINE

Tissue	Potassium Iodide		Iodopropylidene Glycerol	
	Dose/Gm of Tissue, %	Dose in Entire Tissue, %	Dose/Gm of Tissue, %	Dose in Entire Tissue, %
Distribution 4 Hr. after Administration				
Blood, whole	0.31 ± 0.03	5.2 ^a ± 0.5	0.19 ± 0.05	3.3 ^a ± 0.9
Feces, total				
Thyroid, thyroxin	0.025 ± 0.025	0.0004 ± 0.0005	0.68 ± 0.5	0.016 ± 0.008
Thyroid, non-thyroxin	2.5 ± 0.6	0.040 ± 0.012	13.2 ± 4.4	0.25 ± 0.10
Urine, total		18.9 ± 5.7		8.1 ± 4.4
Distribution 12 Hr. after Administration				
Blood, whole	0.078 ± 0.03	1.4 ^a ± 0.5	0.075 ± 0.04	1.6 ^a ± 0.9
Feces, total		28.1 ± 11.		22.4 ± 13.4
Thyroid, thyroxin	0.44 ± 0.25	0.011 ± 0.006	5.4 ± 2.1	0.090 ± 0.040
Thyroid, non-thyroxin	5.9 ± 2.5	0.16 ± 0.08	55.0 ± 14.0	0.91 ± 0.23
Urine, total		49.1 ± 10.		29.1 ± 9.2
Distribution 24 Hr. after Administration				
Blood, whole	0.0091 ± 0.0015	0.15 ^a ± 0.03	0.051 ± 0.012	0.92 ^a ± 0.15
Feces, total		22.1 ± 10		29.7 ± 6.7
Thyroid, thyroxin	4.0 ± 0.8	0.016 ± 0.003	5.9 ± 2.7	0.098 ± 0.042
Thyroid, non-thyroxin	28.5 ± 3.0	0.58 ± 0.09	52.9 ± 17	0.83 ± 0.47
Urine, total		67.4 ± 13.1		35.7 ± 6.4
Distribution 48 Hr. after Administration				
Blood, whole	0.0010 ± 0.00015	0.018 ^a ± 0.0028	0.0083 ± 0.0045	0.15 ^a ± 0.08
Feces, total		22.2 ± 11.1		39.0 ± 14.3
Thyroid, thyroxin	1.67 ± 0.33	0.034 ± 0.014	6.35 ± 3.3	0.11 ± 0.06
Thyroid, non-thyroxin	15.0 ± 2.9	0.30 ± 0.15	52.9 ± 26	0.93 ± 0.40
Urine, total		64.6 ± 12.5		53.9 ± 15
Distribution 72 Hr. after Administration				
Blood, whole			0.0012 ± 0.0019	0.021 ^a ± 0.035
Feces, total				37.7 ± 9.8
Thyroid, thyroxin			3.8 ± 2.0	0.065 ± 0.034
Thyroid, non-thyroxin			28.2 ± 19.0	0.47 ± 0.30
Urine, total				55.0 ± 10.1

^a Based on average blood volume of 6.7 ml per 100 Gm in rats

DISCUSSION

The absorption of 2 IPG from the gastrointestinal tract of the intact rat was found to proceed to the extent of 77% at the end of two hours, (Fig. 2), allowing the conclusion that the compound is well absorbed from an oral dose. It is also shown that the gastrointestinal tract and tissues remained substantially

free of iodide ion during most of this period, indicating that no significant decomposition of 2 IPG, with release of its iodine component, occurred. Since the stomach and intestinal tissues were included in this study, it is considered that the iodine is transported to the blood stream in organic form. The appearance of iodide ion at the one hundred-twenty-minute period, whereas none was observed at the ninety-minute

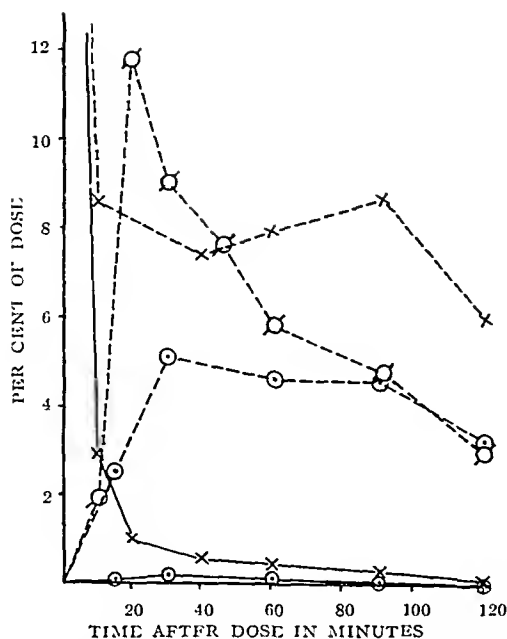


Fig. 1.—Comparison of whole blood content of total iodine and of iodine in IPG following oral (O) and intravenous (x) doses of IPG, and of iodine following oral potassium iodide (φ). Broken lines represent total iodine, solid lines represent iodine in IPG

TABLE II—PER CENT OF DOSE IN RAT ORGANS AFTER INTRAVENOUS INJECTION OF 2 MG OF IODO-PROPYLIDENE GLYCEROL, AVERAGE VALUES OF DATA OBTAINED ON 5 RATS

At 30 Min. after Injection		
Tissue	Per Gram	Per Tissue
Blood, total iodine	0.478%	7.70%
	± 0.037	± 0.60
IPG iodine	0.082	1.61
Brain, total iodine	0.233	0.424
IPG iodine	0.042	0.077
Fat, total iodine	0.291	
IPG iodine	0.231	
Intestine, sm., total iodine	0.945	8.22
IPG iodine	0.054	0.498
Kidneys, total iodine	0.713	1.50
IPG iodine	0.015	0.030
Liver, total iodine	0.533	5.43
IPG iodine	0.014	0.148
Lungs, total iodine	0.311	0.416
IPG iodine	0.031	0.042
Muscle, total iodine	0.274	
IPG iodine	0.048	
Spleen, total iodine	0.342	0.774
IPG iodine	0.019	0.042
Stomach, total iodine	0.755	2.49
IPG iodine	0.053	0.173
At 10 Min. after Injection		
Blood, total iodine	0.537%	8.60%
	± 0.025	± 0.39
IPG iodine	0.155	2.48
Liver, total iodine	0.877	8.07
	± 0.046	± 0.62
IPG iodine	0.033	0.303
	± 0.008	± 0.058

period, is considered to be evidence of secretion of iodide ion back into the tract through the stomach or bile. The results indicated that potassium iodide is more rapidly absorbed than is IPG.

The calculated fraction in the blood of the dose of IPG was always small, even only 2% at ten minutes after the intravenous dose, and always small compared with the total blood iodine. These facts require that the IPG be efficiently and quickly removed from the blood stream, and that metabolic release of the iodine from its organic combination be commenced immediately. Direct metabolic release of the organic iodine to iodide so rapidly by the blood or organs seems contraindicated by the long iodide blood levels relative to those from potassium iodide. Absence of IPG from the urine and the long iodide levels would seem to rule out excretion.

No organ or tissue studied at any of the time intervals after administration of IPG revealed any storage of either IPG or total iodine which could be considered significantly large in relation to the dose. The fat showed an early storage of IPG significantly above the total iodine level of the blood, at the same time period (thirty minutes). Since IPG is soluble in fats, the fat might be considered a logical storage depot. However, the iodine levels in the fat were barely detectable at the four-hour period (Table I) and were not detected at longer time intervals. Such a short storage time seems incompatible with the duration of blood levels observed.

Although total iodine levels in the kidneys, small intestine, stomach, and liver thirty minutes after the dose (Table II) were significantly above that in the blood at the same time, the total-organ content of iodine in each case was insignificant in comparison with the dose, and the idea of these tissues being

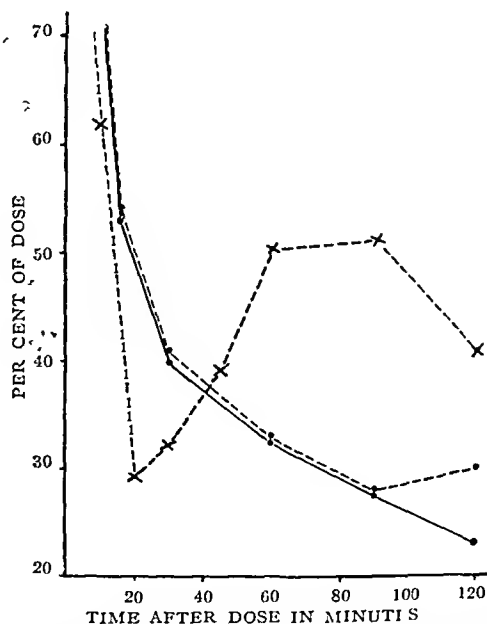


Fig. 2.—Remaining total iodine (broken line) and iodine in IPG (solid line) found in gastrointestinal tract of rats following oral doses of IPG (•) and of potassium iodide (x).

possible storage sites for IPG or iodine metabolized from it was discarded

For its size, the thyroid gland is capable of storing a large amount of iodide. However, the amounts observed cannot be considered as representing significant storage in comparison to the total therapeutic dose, and the chromatograms did not detect any IPG.

The possibility exists that the acetal portion of IPG could be broken enzymatically to release iodinated three-carbon residues, which might escape detection as organic iodine by means of chloroform extracts because of higher water solubility. However, this same alteration of solubilities should have resulted in the appearance of separate entities in the paper chromatographic studies, but no reproducible evidence of separate entities was obtained. This was not construed to mean that the acetal ring is not broken in the metabolism of the compound. It merely gave some evidence that, if this does occur, it is probably after the iodine has been removed.

The possibility exists of the release of iodine from IPG in elemental form by a metabolic process of general distribution in the body, with consequent rapid combination with body proteins. This could be sufficiently widespread to be unobservable as a storage in a specific tissue. Also, widespread low-concentration storage of IPG itself in these tissues, with relatively slow release of iodide, is possible. These two mechanisms seem to be reasonable explanations for the data obtained in the investigation.

The investigation of iodine storage in the thyroid gland indicated that, following a single therapeutic dose, the gland was able to trap more iodine from IPG than from potassium iodide, was able to convert more of the iodide to thyroxine form from the IPG dose, and able to maintain these higher iodine levels over the comparison period studied. The investigations also revealed that the thyroid glands did not trap IPG as such, but trapped iodide released from IPG. The blood iodide levels alone, compared with those of potassium iodide, do not constitute a reason-

able explanation of this picture. The possibility that this effect was the result of the use of a 60% glycerin solution as the vehicle for IPG exists, and further work on this point is indicated.

From the prolonged duration of blood levels of IPG following a single dose, it might be presumed that in a course of therapy continuously effective thyroxine-blocking levels of blood iodide would be produced by IPG. Also, it might be considered that these levels would be maintained with IPG at a lower daily dosage than would be necessary with potassium iodide.

It might be noted here that McKnight (1, 2) produced successful involution of hyperthyroid glands and rapid cessation of thyrotoxic symptoms in the patients with large (300 mg.), once-daily, intravenous doses of IPG, without untoward symptoms.

Comparison of data obtained in this investigation with those of Broking (12), Bonanni (13), and Forbes (14), on the rate of release of iodine from iodized fatty acids and fats indicates that IPG releases iodine more rapidly than these compounds, and seems, therefore, to be intermediate between them and inorganic iodine compounds with respect to the rate of availability of its iodine.

REFERENCES

- (1) McKnight, R. B., *N. Carolino Med. J.*, 7, 590 (1946)
- (2) McKnight, R. B., *Southern Surgery*, 13, 810 (1947)
- (3) Slaughter, D., Belogorsky, J. B., and Schwartz, C., *Amer. Therop. Soc. Meeting*, Boston, April 13-16, 1950
- (4) Slaughter, D., *S. Dakota J. Med. and Pharm.*, 1, 425 (1948)
- (5) Information supplied by Henry K. Wampole & Co. Inc., Phila., Pa.
- (6) Brochure, "Organidin®," Henry K. Wampole & Co. Inc., Phila., Pa.
- (7) Brown, H. B., and Page, I. H., *Circulation*, 10, 714 (1954)
- (8) Blau, N. F., *J. Biol. Chem.*, 110, 351 (1935)
- (9) Owen, C. A., and Power, M. A., *ibid.*, 200, 111 (1933)
- (10) Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, 111, 605 (1950)
- (11) Hoffnagle, G. F., Siders, C. N., and Osol, A., "Paper Chromatography of I¹³¹-Labeled with Autoradiographic Identification"
- (12) Broking, E., *Z. Exptl. Path.*
- (13) Bonanni, A., *Zentr. Biochem. u. Biophys.*, 15, 120 (1943)
- (14) Forbes, J. C., *THIS JOURNAL*, 37, 509 (1948)

Book Notices

A Glossary of Mycology. By WALTER H. SNELL and ESTHER A. DICK. Harvard University Press, Cambridge, Mass., 1957. xxvi + 171 pp. 17 x 26 cm. Price \$5.

This dictionary includes among its 7,000 terms some which are not strictly mycological but might be useful to students because they do occur in mycological literature. Definitions referring to the fungi include technical, popular, vernacular, and obsolete terms; terms used in medical mycology and antibiotics; folklore, and color terms; and names of the originators of the terms. The type is small, but this is typical of books in this and related fields. It should be a useful reference for

teachers, advanced students, and amateur and professional mycologists.

Dorland's Illustrated Medical Dictionary. 23rd ed. W. B. Saunders Company, Philadelphia, 1957. xvii + 1,598 pp. 17 x 26 cm. Price \$12.50.

This new edition of Dorland's is bigger and, if possible, better than ever. Accuracy, authority, and quick usefulness remain the principal objectives of this reference work. It has been reviewed, revised and modernized. It is as new as the recently coined "atacetic" and "ataraxic," with their blurred difference; the respective definitions being: "Per-

taining to or capable of inducing ataraxia," and "An agent capable of inducing ataraxia." The fact that Austin Smith is responsible for modern drugs and dosage is assurance to physicians and pharmacists that expert attention has been given to this important phase of the dictionary. The longer page has permitted the addition of many words, without making the book bulkier, and the stiffer backing is a protective aid for standing usage.

Dictionary of Microbiology. By MORRIS B. JACOBS, MAURICE J. GERSTEIN, and WILLIAM G. WALTER. D. Van Nostrand Company, Inc., Princeton, N. J., 1957. 276 pp. 15 x 23.5 cm. Price \$6.75.

This book contains more than 5,000 items of interest to bacteriologists, cytologists, immunologists, mycologists, biochemists, and others in related medical fields. More detailed entries are included for important culture media and methods, stains and staining, and diagnostic tests. Other entries include types of apparatus, names of chemical substances, enzymes, etc. Although some drugs are given by their generic names, and trade names are referred to the generic names, the trade names are not marked and many are omitted; e. g., under Tetracycline. . . "The group of tetracyclines, including oxytetracycline (Terramycin), chlorotetracycline (Aureomycin), tetracycline (Achromycin), Chlorotetracycline should be chlortetracycline; and, for tetracycline, the trade names Panmycin, Polycycline, Steclin, and Tetracyn are omitted. However, the book should be a useful addition to the reference shelf for the many items which it includes that are not concisely described in other books; e. g., BCG Vaccine is defined, and another entry defines Calmette and Guérin's Bacillus with a cross-reference to BCG.

Heterocyclic Compounds. Vol. 6. Edited by ROBERT C. ELDERFIELD. John Wiley & Sons, Inc., New York, 1957. vii + 753 pp. 15 x 23.5 cm. Price \$25.

This volume, the sixth in this authoritative and comprehensive series, covers the chemistry of the six-membered heterocycles containing two hetero atoms and their benzo derivatives, with the parent monocycles being treated separately from their benzo derivatives. In general, the attempt has been made to cover the major English and German language periodicals through 1955.

The text material is divided into chapters, contributed by specialists, under the following headings: the chemistry of the monocyclic dioxanes; benzo-dioxanes; sulfur analogs of the dioxanes; pyridazines; cinnolines and related compounds; phthalazines and its derivatives; pyrimidine and its derivatives; the chemistry of quinazoline; the pyrazines and piperazines; the quinoxalines; the monocyclic oxazines; the benzoxazines; thiazines and benzo-thiazines; phenazines, phenoxazines, and phenothiazines. The clear style, format, and type of the earlier volumes are continued in volume 6, and all that has been said in praise of this series can be repeated with much more reason now. No chemical library can be considered complete without this reference work.

The Chemistry of Plants. By ERSTON V. MILLER. Reinhold Publishing Corp., New York 22, N. Y., 1957. vii + 174 pp. 15 x 23 cm. Price \$4.75.

This book presents a very limited coverage of the chemical substances found in plants, with brief descriptions of their composition, biochemical reactions, and physiological behavior. The information is presented in an interesting manner. Alkaloids are covered in a 15-page chapter which includes a discussion of the Rauwolfia alkaloids; mentioning that *R. serpentina* has been found to contain 14 substances of alkaloidal nature [more than 20 alkaloids isolated from *R. serpentina* were reported in THIS JOURNAL, 44, 553 (1955)] and noting that "Structurally reserpine contains a pentacyclic ring system." The text gives general references after each chapter, and a subject index is appended. The book should be a useful reference for those who do not wish extensive and detailed information in this field.

Experiments in Biochemical Research Techniques. By ROBERT W. COWGILL and ARTHUR B. PARDEE. John Wiley & Sons, Inc., New York, 1957. ix + 188 pp. 15 x 23 cm. Price \$3.50.

This book is a laboratory text that includes thirty-seven experiments that are intended to illustrate some of the major research techniques of modern biochemistry. Physical chemical methods include: Distillation at low pressures, Countercurrent distribution, Chromatography, and Zone electrophoresis. Seventeen experiments are related to the biochemistry of enzymes. Seven experiments illustrate radioisotope tracer techniques in biochemical research. Special laboratory exercises are appended and a subject index is included. The book represents a good printing and binding job and should be a useful addition to the research library and helpful to the teacher of graduate students.

Principes de Synthèse Organique. Introduction au Mécanisme des Réactions. Edited by LÉON VELLUZ. Masson et Cie., Éditeurs, Paris, 1957. x + 599 pp. 15 x 22 cm. Price 6,000 fr.

This book (in French) considers the theoretical principles of organic chemistry as they are related to syntheses and other reactions. The first four parts of the text cover Electronic factors, Mechanisms of reactions, Steric factors, and Steric aspect of reactions. Part five includes discussions on: Degrees of oxidation, Influence of the solvent, Hammett's equation for quantitative electronic effects; and gives tabulations of: Organic groups, Radicals, and Compounds with various properties and constants. A good subject index is appended and an extensive table of contents is at the end of the book.

International Encyclopedia of Cosmetic Material Trade Names. By MAISON G. DENAVARRE. Moore Publishing Company, New York, 1957. lvi + 359 pp. 16 x 23 cm. Price \$7.50.

This book is a useful compilation of information about materials and compositions in the field of cosmetics. The close relation of dermatological

formulations with many cosmetic ingredients makes the book a good reference for pharmacists. The text is divided into a main section, which lists materials by trade names; a product classification section (noted with each entry in the main listing); and a directory of manufacturers.

Organic Reactions. Vol. IX. ROGER ADAMS, Editor-in-Chief. John Wiley & Sons, Inc., New York, 1957. vii + 468 pp. 15 x 23 cm. Price \$12.

This ninth volume of a series of organic reactions continues the excellent comprehensive reviews that started with volume I in 1932. Volume VIII was reviewed in *THIS JOURNAL*, 44, 128(1955). Contributions of chapters by authors with special knowledge of the particular reactions is continued, as is the general plan of presentation followed in the earlier volumes.

The reactions included in this volume are indicated by the chapter headings: The cleavage of non-enolizable ketones with sodium amide, The Gattermann synthesis of aldehydes, The Baeyer-Villiger oxidation of aldehydes and ketones, The alkylation of esters and nitriles, The reaction of halogens with silver salts of carboxylic acids, The synthesis of β -lactams, and The Pschorr synthesis and related diazonium ring closure reactions. The reference value of the work is aided by tabulations of compounds that have been prepared by or have been involved in a particular reaction. The text is documented with many references to the literature and a subject index is appended. Teachers, advanced students, synthetic chemists, and libraries should have access to this book, and those who have used the earlier volumes will welcome this addition to the series.

Apotekens Register över Standardförpackade Lakemedel 1957. Edited by Apotekarsocietetens Laboratorieavdelning. Victor Pettersons Bokindustri Aktiebolag, Stockholm, Sweden, 1957. xix + 481 pp. 17 x 23 cm.

This useful reference for proprietary drugs, particularly those of Scandinavian manufacture, is published annually with paste-in supplements made available during the year. In addition to its value in a reference library, the book is a beautiful example of the art of printing and binding.

Phosphorus and Fluorine, The Chemistry and Toxic Action of Their Organic Compounds. By BERNARD CHARLES SAUNDERS. New York: Cambridge University Press, New York, 1957. xiii + 231 pp. 14 x 21.5 cm. Price \$5.

This monograph is directed mainly to the advanced student of chemistry and the industrial chemist, particularly those who manufacture and handle toxic organic compounds of phosphorus and fluorine. These compounds find applications as chemical warfare agents, as constituents of agricultural insecticides, in enzyme system investigations, and as medicinal agents. The author writes with authority, particularly about di-isopropyl phosphoro-fluoridate (D. F. P.), a typical "nerve gas," the discovery of which is accredited to him.

Particularly interesting to pharmacists is Chapter

X which covers esterase activity and medical aspects. The latter includes the use of D. F. P. in postoperative paralytic ileus and its effect in myasthenia gravis, and the radiosensitizing effect of organic phosphates in the radiotherapy of malignant tumors. A concise statement on first-aid treatment for nerve-gas poisoning stresses the use of atropine injection (2 mg.) repeated at intervals and artificial respiration.

The usefulness of the monograph is enhanced by having cited references as footnotes in the text. Added appendices include methods of determination of fluorine in organic compounds and a table of properties of typical fluoro compounds. A general bibliography and a subject index are included. The format, type, and binding of the book are first rate. Medical, pharmaceutical, and chemical libraries should add this monograph to their collections.

Regulation and Mode of Action of Thyroid Hormones. Ciba Foundation Colloquia on Endocrinology. Edited G. E. W. WOLSTENHOLM and ELAINE C. P. MILLAR. Little, Brown and Company, Boston, 1957. xii + 327 pp. 13.5 x 20.5 cm. Price \$8.50.

The papers compiled in this volume attempt to review the developments during the last ten to fifteen years on concepts of regulation of the thyroid gland and the character and mode of action of its hormones. The subjects presented include: Hypothalamus-pituitary-thyroid relationships; Regulation of thyroid activity; Influence of the central nervous system on the control of thyrotrophin secretion; Pituitary cytology and thyrotrophic hormone secretion; Factors influencing the thyroidal iodide pump; the "feed-back" hypothesis of the control of thyroid function; Hormone synthesis in the iodine-deficient thyroid gland; Enzymic aspects of thyronine metabolism and its iodinated derivatives; the metabolism of thyroid hormones by kidney and the biological activity of the products; The distribution and metabolism of thyroid hormones; Hepatic regulation of thyroxine metabolism; Thyroid hormones at the peripheral tissue level: metabolism and mode of action; Cellular actions of thyroxine and similar compounds; some observations of the clinical effects of triiodothyroacetic acid; and The influence of the thyroid gland upon immune responses of different species to bacterial infection. References are given after each paper and the appended subject index adds to the reference value of this volume.

Travaux des Laboratoires de Matière Médicale et de Pharmacie Galénique de la Faculté de Pharmacie de Paris. Vol. XLI, 1956. Edited by M.-M. JANOT and R. PARIS. Vigot Frères, Éditeurs, Paris, 1957. 15.5 x 23.5 cm. 479 pp.

This book (in French) is a compilation of original articles and theses on the subjects: Auto-injectable ampuls of the military health service; Contribution to the study of the alkaloids and flavonoids of some indigenous Genistas with the aid of electrophoresis; Studies on some African Rutaceae with alkaloids of the Fagara family; Contribution to the study of *Aphloia theaeformis*; Identification of drugs and control of medicaments of vegetable origin by paper

chromatography. Abstracts of other articles are included in the poorly printed, paper-back volume. No index is given.

Catalysis in Practice. Edited by CHARLES H. COLLIER. Reinhold Publishing Corporation, New York, 1957. v + 153 pp. 15 x 18.5 cm. Price \$3.95.

Actual methods, economics, and problems of catalysis in the chemical process industries are described. Included are chapters on catalyst choice and commercial preparation; fixed bed systems and moving bed processes; economics of catalyst use; operating problems in processing; and an appraisal of trends and prospects in catalysis.

The Clinical Application of Antibiotics Vol. III, *Chloramphenicol and the Tetracyclines.* By M. E. FLOREY. Oxford University Press, New York, 1957. ix + 393 pp. 15 x 25 cm. Price \$19.50.

This volume is a continuation of the author's earlier work "The Clinical Application of Antibiotics—Penicillin" which was published in 1952 and which must now be considered as Volume I of this series. The aim of this book is to evaluate critically the data on which the present use of chloramphenicol and the tetracyclines is founded. The evidence in this volume on which clinical practice is based will help to make general principles clearer. Methods of treatment are given in detail. The importance of accurate diagnosis is stressed.

Bergey's Manual of Determinative Bacteriology. 7th ed. By ROBERT S. BREED, E. G. D. MURRAY, NATHAN R. SMITH, *et al.* The Williams and Wilkins Company, Baltimore, 1957. xviii + 1,094 pp. 15 x 23 cm. Price \$15.

The format of this edition differs but little from that of the sixth edition. Many changes in the content, however, as the result of a thorough-going revision are apparent. Much historical material in the sixth and earlier editions of the Manual has been excluded. The new edition represents the coordinated results of the work of 100 contributors.

Methods of Biochemical Analysis. Vol. V. Edited by DAVID GLICK. Interscience Publishers, Inc., New York, 1957. ix + 502 pp. 15 x 23 cm. Price \$9.50.

This book is designed to present chemical, physical microbiological, and if necessary, animal assays; as well as basic techniques and instrumentation for the determination of enzymes, vitamins, hormones, lipids, carbohydrates, proteins and their products, minerals, antimetabolites, etc.

Biochemistry and Human Metabolism. 3rd ed. By BURNHAM S. WALKER, WILLIAM C. BOYD, and ISAAC ASIMOV. The Williams and Wilkins Company, Baltimore, 1957. vii + 937 pp. 15 x 23 cm. Price \$12.

The main effort in preparing this edition has been

to keep the text as nearly as possible abreast of the field indicated by the title. Parts of the text have been rewritten and re-arranged and much new material has been introduced.

Catalysis. Vol. V. Edited by PAUL H. EMMET. Reinhold Publishing Corporation, New York, 1957. vi + 542 pp. 15 x 23 cm. Price \$15.

This fifth volume includes reactions of carbon monoxide, the oxo reaction, the direct catalytic synthesis of higher alcohols from carbon monoxide and hydrogen, the catalytic hydrogenation of aromatic compounds, hydrogen isotopes in the study of hydrogenation and exchange, and the hydrodesulfurization of liquid petroleum fractions. Subsequent volumes will deal with halogenation, isomerization, alkylation, polymerization, cracking, decomposition, and miscellaneous reactions.

The Chronically Ill. By JOSEPH FOX. The Philosophical Library, Inc., New York, 1957. xix + 229 pp. 13.5 x 21.5 cm. Price \$3.95.

The material contained herein is of special interest to the physician, the social worker, the hospital administrator, and to labor and management. This book is a sociological approach to the problem of conquering the ravages of chronic diseases.

Microtechniques of Clinical Chemistry for the Routine Laboratory. By SAMUEL NATELSON. Charles C Thomas, Publisher, Springfield, Ill., 1957. xv + 484 pp. 15 x 23 cm. Price \$11.

This book is written for investigators interested in following the effect of disease or drugs on the human organism or small laboratory animals. It is also of value to those faced with analysis of inorganic and organic constituents present in small amounts in all fields of chemistry. The basic principles used in microanalysis and the equipment needed for this purpose are discussed.

Complexometric Titrations. By GEROLD SCHWARZENBACH. Translated and revised in collaboration with the author by Harry Irving. Interscience Publishers, Inc., New York, 1957. xviii + 132 pp. 14 x 22 cm. Price \$3.75.

The author has presented a clear and full account of the basic theory in such a way that analysts may be able to plan new determinations and to understand the reasons for each step in the detailed procedures. The book contains a full account of the theory and practice of metal-indicators. The preparation of standard solutions of complexones and the necessary buffers and indicators are described. Full detailed accounts of specific determinations are given.

Digitalis. Compiled and edited by E. GREY DIMOND. Charles C Thomas, Publisher, Springfield, Ill., 1957. xiv + 255 pp. 15 x 23 cm. Price \$7.

Collected in this book are the present day concepts of the use of digitalis, up-to-date ideas of dosage, treatment of intoxication, and preparations of choice.

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**The Separation and Determination of Reserpine,
Deserpidine, and Rescinnamine
By Partition Column Chromatography***

By ALMA L. HAYDEN, LEONARD A. FORD, and ALBERT E. H. HOUK

A method is proposed for the column chromatographic separation of deserpidine, reserpine, and rescinnamine, and their determination by ultraviolet spectrophotometry. The method is useful for determining the purity of the crystalline alkaloids, and is suitable for the assay of their commercial mixtures.

IN THE COURSE of investigations in this laboratory on Rauwolfia alkaloids in therapeutic preparations, the need of a method for the quantitative separation of reserpine, deserpidine, and rescinnamine became apparent. This need was heightened by the lack of absolutely pure specimens of the three alkaloids. All available crystalline samples of the three substances were found to contain small amounts of one or both of the other congeners.

Carol and his collaborators (1, 2) have developed a qualitative procedure for separating reserpine, deserpidine, and rescinnamine by paper chromatography, using an isoöctane-carbon tetrachloride-piperidine-tertiary butyl alcohol-formamide system. The procedure presented here separates the three alkaloids quantitatively by liquid-liquid partition column chromatography. The alkaloids are then determined by direct ultraviolet spectrophotometry of the eluates in the

region between 260 and 330 $m\mu$. The solvent system is *n*-heptane-chloroform-morpholine-formamide. Celite® 545 as obtained is used as the solid supporting material.

EXPERIMENTAL

Materials.—Solvents—(a) U. S. P. chloroform and methanol; redistilled *n*-heptane and morpholine. On comparison with water at 272 $m\mu$, the optical density values of these solvents should be less than 0.200.

(b) Formamide. Distill in all glass apparatus under vacuum. Reject the first portion of distillate containing water, and collect the fraction which boils at about 101°/12 mm. Hg (115°/25 mm. Hg).¹ Store the purified formamide over concentrated sulfuric acid to remove most of the ammonia. The formamide should be neutral or slightly alkaline.

Diatomaceous Silica Support—Celite® 545, produced by the Johns-Manville Co.

Eluent—Mix thoroughly, in a separatory funnel, 715 ml. of *n*-heptane, 110 ml. chloroform, 1 ml. of morpholine, and 25 ml. of formamide. After equilibration (about 20 minutes) separate the formamide layer and retain it for use as the immobile solvent. Filter the eluent (top layer) through CHCl_3 -washed cotton. Prepare the solvent system on the day of use.

¹ Some commercial formamide contains a strongly absorbing impurity which is not removed completely on distillation under the above conditions. Adsorption chromatography on Fisher alumina removes this impurity sufficiently to allow the direct use of the filtered eluate for spectrophotometry. However, many formamide distillates with optical densities up to 1.3 at 272 $m\mu$, when compared with water in 1 cm. silica cells, were used without apparent effect upon the absorption of the eluates or the resolution of the alkaloids.

* Received December 26, 1957, from the Division of Pharmaceutical Chemistry, Bureau of Biological and Physical Sciences, FDA, Department of Health, Education, and Welfare, Wash., D. C.

Preparation of Standard Solutions of Reserpine, Deserpidine, and Rescinamine.—Accurately weigh about 1 mg. of each alkaloid. Dissolve each sample in a few drops of chloroform and dilute to 100 ml. with the filtered eluent. Measure the absorbances of these solutions between 260 μ and 330 μ in a suitable spectrophotometer relative to the eluent as blank. Since there is some decomposition of these alkaloids on standing in the eluent, the readings should be made immediately after preparation of the solutions. In the concentration range studied (up to 0.020 mg./ml.) solutions of these alkaloids in eluent and eluate follow Beer's law at their respective absorption maxima. To permit direct reading of the eluates in the ultraviolet spectrophotometer, the use of the eluent as reference blank is necessary.

Chromatographic Tube.—A 350 mm. length of glass tubing (i. d. 21 mm.) fused to a piece of tapered outlet tubing.

Preparation of the Column.—Place a small pledget of CHCl_3 -washed cotton in the bottom of the chromatographic tube. Thoroughly mix 25 Gm. of Celite and 20 ml. of the immobile solvent. Add this mixture to the tube through a powder funnel in five equal portions by weight and tamp each portion tightly to a height of 4.8 cm. Add about 0.5 Gm. of Celite to the column and tamp down tightly.

Preparation of the Samples.—Dissolve an accurately weighed sample² containing 0.5–5.0 mg. of the alkaloid(s) in about 1 ml. of chloroform-methanol (3 + 1) and transfer to a 30-ml. beaker containing 1 Gm. of Celite, with the aid of several small portions of chloroform-methanol. Mix thoroughly and allow the solvent to evaporate under a hood fan or under vacuum at room temperature.

Add the sample-Celite mixture to the prepared column using about 0.5 Gm. of Celite to rinse the sample beaker. Add a small pledget of CHCl_3 -washed cotton to the top of the column and tamp down tightly. Rinse the sample beaker three times with about 2 ml. of eluent and add the rinsings to the column.

Place the remainder of the eluent in a stoppered separator as reservoir, and allow the eluent to percolate through the column. Adjust the level of liquid above the column to yield an initial flow rate of about 65–70 drops of eluate per minute (5 ml. in three minutes). Develop the chromatogram at 23°–26°.

In general, for amounts up to 1.5 mg. of total alkaloids, collect a 100-ml. forerun; four 5-ml. fractions; deserpidine in the next 75 ml.; five 5-ml. fractions; reserpine in the next 110 ml.; six 10-ml. fractions; rescinamine in the next 200 ml. and finally five 5-ml. fractions. The fractions separating the alkaloids are collected in 5-ml. portions to establish the minimum absorption before and after the elution of each alkaloid, to permit the detection of any trailing between zones, and to detect early elution of the alkaloids. The time required for complete elution of the alkaloids is about six hours.

For amounts greater than 1.5 mg. collect sufficient 5 ml. fractions (on the basis of ultraviolet readings) before and after each main eluate to detect any early elution and trailing due to the larger amount of

sample. For the determination of alkaloids present to the extent of less than 0.50 mg. collect the appropriate fraction in 5-ml. portions of eluate.

Determine the ultraviolet absorption spectra of the various fractions relative to the eluent as blank between 260 μ and 330 μ . Make any necessary adjustments in the observed readings for overcompensation of eluent. The eluates containing deserpidine show a maximum absorption at 272 μ ; reserpine shows maxima at 266–268 μ and 295 μ ; and rescinamine shows a maximum at 303–305 μ . Calculate the quantities of alkaloids in the eluates by comparing their absorbances, corrected for background absorbance, with those of the corresponding standard solutions.

DISCUSSION

The proposed method of analysis was applied to crystalline samples of reserpine, deserpidine, and rescinamine, and to synthetic and commercial mixtures of the alkaloids. The purest samples of the three alkaloids were chosen as the standards. The elution of the three alkaloids in the order of deserpidine, reserpine, and rescinamine is consistent with their relative R_f values in paper chromatograms.

The average recoveries of deserpidine, reserpine, and rescinamine were 99, 97, and 95%, respectively, in the analysis of both individual alkaloids and synthetic mixtures. These recoveries were corrected for the presence of alkaloidal impurities in the standards. One to two mg. of crystalline material was used for the assay of individual alkaloids.

Results of chromatographic analysis by the proposed method are listed in Table I. Recoveries for the reserpine samples are based on direct comparison with the standard solutions. Samples R6 through R10 therefore contain as much pure reserpine as the standard, while samples R1 through R5 are contaminated with appreciable quantities of other alkaloids.

For the detection of very small amounts (1–5%) of one alkaloid in a sample of another, 1–5 mg. of sample was used. Amounts up to 10 mg. of an individual alkaloid can be analyzed for small amounts of the congeners if the concentration of the adjacently eluted congener does not exceed 3%; however, much larger volumes of eluent are required, and considerable trailing between zones occurs. In addition, when these larger amounts are used, the recovery of the main constituent is lowered.

Two per cent rescinamine was indicated in the deserpidine standard. Both deserpidine (1%) and rescinamine (1%) were indicated in the reserpine standard. The rescinamine standard contained 2% reserpine.

Figure 1 shows the complete chromatographic separation of 1.5 mg. of a synthetic mixture containing 33% deserpidine, 33% reserpine, and 34% rescinamine. With concentrations up to about 0.5 mg. of each component of a mixture there is complete separation. With increasing concentrations of one or more of the components, larger volumes of eluent may be required, and considerable trailing of one fraction into an adjacently eluted fraction may occur. However, complete separation is obtained when increasing concentrations of one component is accompanied by decreasing concentrations of the other component(s) (Samples 3 and 4). Some crys-

² Aliquots of alcoholic solution of the alkaloids may be concentrated in vacuo to give the desired amount of sample.

TABLE I.—CHROMATOGRAPHIC ANALYSIS OF INDIVIDUAL ALKALOIDS AND MIXTURES

Sample Number	Description	Deserpine, %		Reserpine, %		Rescinamine, %	
		Declared or Added	Found	Declared or Added	Found	Declared or Added	Found
1	0.50 mg. Standard Deserpine	100	98	2.0
2	1.956 mg. Standard Reserpine	..	0.85	100	96.0	..	1.3
3	1.760 mg. Standard Rescinamine	2.2	100	94.4
4	Synthetic mixture of 0.098 mg. Deserpine and 2.7 mg. Reserpine +0.095 mg. Rescinamine	4.14	4.04	91.8	90.0	4.01	3.9
5	Synthetic mixture of 0.098 mg. Deserpine +0.122 mg. Reserpine +1.65 mg. Rescinamine	5.24	5.24	6.52	6.5	88.0	90.0
6	1.0 mg. Commercial mixture M1	25.0	25.5	75.0	73.0
			24.6		72.3	..	2.3
7	1.1 mg. Commercial mixture M2	20.0	20.2	60.0	59.8	20.0	19.6
8	2.0 mg. Commercial Reserpine R1	0	14.1	100	87.8	0	0.0
9	2.0 mg. Commercial Reserpine R2	0	3.7	100	92.6	0	0.0
10	2.5 mg. Commercial Reserpine R3	0	9.8	100	74.8	0	3.2
11	4.0 mg. Commercial Reserpine R4	0	5.7	100	89.0	0	..
			5.7		90.5		1.1
12	5.1 mg. Commercial Reserpine R5	0	4.2	100	87.2	0	4.7
13	2.0 mg. Commercial Reserpine R6	0	1.2	100	95.7	0	0
14	2.0 mg. Commercial Reserpine R7	0	1.4	100	95.9	0	0
15	2.0 mg. Commercial Reserpine R8	0	0.6	100	96.1	0	0
16	2.0 mg. Commercial Reserpine R9	0	0.5	100	95.9	0	..
17	2.0 mg. Commercial Reserpine R10	0	1.0	100	95.7	0	0.8
18	2.0 mg. Commercial Deserpine D1	100	95.1	0	2.7	0	0
19	2.0 mg. Commercial Deserpine D2	100	89.0	0	7.1	0	0.8
20	2.0 mg. Commercial Deserpine D3	100	93.8	0	3.8	0	0

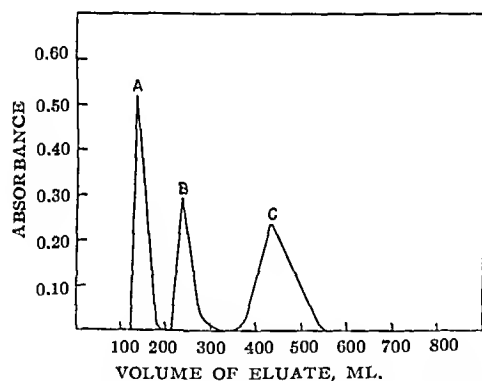


Fig. 1.—Chromatographic Separation of Deserpine (A), Reserpine (B), and Rescinamine (C).

talline reserpine samples, containing 1 to 14% deserpine and 1 to 5% rescinamine were analyzed in amounts up to 2 mg. with excellent results.

The elution pattern of the alkaloids varies when the experimental conditions are altered. The use of a larger proportion of formamide and/or the omission of morpholine in the solvent system results in lower recoveries of the alkaloids. A larger proportion of chloroform results in earlier elution and poor separation of the alkaloids. Increase or decrease in the amount of Celite alters the volumes of eluent required, the time of elution, and the resolution of the alkaloids. An increase in flow-rate decreases the resolution and the time of elution.

Under the given experimental conditions the volume of eluent required and the position of each alkaloid in the elution pattern are reproducible within the optimum concentration ranges. When these condi-

tions do not exist, it is necessary to track the chromatogram by analysis of smaller cuts.

Table II presents a comparison of results obtained for crystalline reserpines by column chromatography, by semi-quantitative paper chromatography (1, 2), and by the nitrite procedure (3). The excellent

TABLE II.—ANALYSIS OF CRYSTALLINE RESERPINE BY VARIOUS METHODS

Sample	Deserpine, %		% Reserpine Plus Rescinamine	
	Paper	Proposed Method	Nitrite ^a	Proposed Method
R1	Over 8%	14.1	85.0	87.8
R2	About 4%	3.7	93.8	92.6
R3	Over 8%	9.8	77.7	78.0
R4	Over 4%	5.7	94.6	91.6
R5	About 4%	4.2	..	91.9
R6	Less than 2%	1.2	..	95.7
R7	Less than 2%	1.4	..	95.9
R8	Less than 2%	0.6	98.8	96.1
R9	Less than 2%	0.5	97.3	95.9
R10	Less than 2%	1.0	96.3	96.5

^a This method does not differentiate between reserpine and rescinamine.

agreement among these independent methods indicates that all of them are useful for the analysis of reserpine preparations. Investigations on the application of the proposed method to the analysis of Rauwolfia root extracts will be reported elsewhere.

REFERENCES

- (1) Banes, D., Carol, J., and Wolff, J., *THIS JOURNAL*, 44, 640(1955).
- (2) Carol, J., private communication.
- (3) Banes, D., Wolff, J., Fallscheer, H. O., and Carol, J., *THIS JOURNAL*, 45, 710(1956).

Trichome Variability in Commercial Sages*

By H. I. SILVERMAN and A. URDANG

A quantitative ratio of the number of trichomes occurring in a definite weighed amount of dried sage leaf has been determined. A mean has been calculated upon which further analyses of sage in foods can be based. Also, quantitative measurements of the basal cells of seven samples of sage have been tabulated.

IN A QUANTITATIVE microanalytical study recently undertaken (1), it was noted that the nonglandular uniseriate hairs of Dalmatian sage varied in number from lot to lot in samples studied. Accordingly several batches of *Salvia officinalis* Linn. occurring on the market were purchased and microscopically analyzed for number of trichomes per weight of dried leaf.

It was thought that a permanent record (2) of the variation and number of trichomes per weight of dried leaf, or drug, would be valuable for microanalytical and/or quantitative plant work.

Microscopically the leaf of *Salvia officinalis* Linn. is covered on both its upper and lower epidermis with nonglandular uniseriate hairs, one- to five-celled and generally curved. The end cell is attenuated and the basal cell thicker in its walls with a narrower lumen (Fig. 1). Upon comminution of sage leaves the hairs are broken up into varying lengths; however, the basal cells remain intact, probably due to the greater thickness and consequently increased strength of their walls as compared to the other trichome cells.

After extended investigation it was found that these basal cells, in a ground sample, could be utilized for a counting procedure in which the number of trichomes per weight of comminuted leaf was determined.

METHOD

The samples were first identified by suitable macro and microscopical methods and their commercial designations verified. A representative sample of each of these purchased samples was then ground sufficiently fine to pass entirely through a number 80-mesh sieve and then treated as follows in order to aid the microscopist in his recognition of the basal cells during the counting procedure.

The ground sage was treated with ether (at the boiling point) to remove interfering pigments, fats, and oils. After removal of the ether, the sample

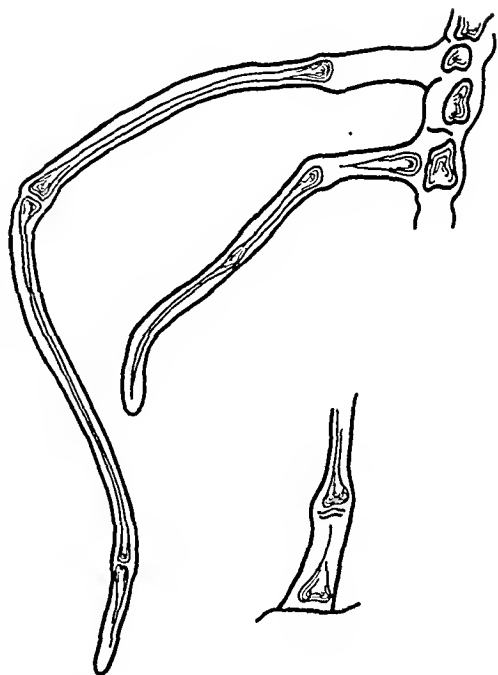


Fig. 1.—Non-glandular trichomes of sage.

was then stained with a 0.5% aqueous solution of Bismarck brown for five minutes. It was then washed with distilled water to remove excess stain and oven dried at 50° (not less than twelve hours) until a constant weight was obtained. The powdered dried sample was thoroughly mixed and a portion whose weight was usually between one-tenth and one milligram was removed and accurately weighed on a microchemical balance. After weighing, the material was carefully transferred to a lucite plate measuring 6 x 5 cm. with a 4-cm. square etched net-like ruling of 1-mm. spaced lines (Fig. 2). A few drops of a 2% sodium carboxymethyl-cellulose¹ gel were placed on the plate, and mixed with the sample, and the plate was then covered with a suitable cover glass. Examination and counting of the basal cells observed was carried out in the usual manner using the etched lines of the lucite plate as an aid in scanning the sample covered area.

A minimum of four separate portions were removed from each of the stained samples and counted.

DATA

Table I illustrates the weights taken of each sample, hairs counted, and hair indices (number of

* Received May 20, 1957, from Long Island University, Brooklyn College of Pharmacy, N. Y.

¹ CMC-70-D High, Hercules Powder Co.

TABLE I.—TRICHOME COUNT OF SAGE LEAVES

Sample No									Av. No. Hairs/mg
1	mg	0	456	0	368	0	447	0	490
	Hairs		349		225		271		254
	Index		765		611		606		518
2	mg.	0	461	0	466	0	265	0	378
	Hairs		322		313		151		189
	Index		698		672		570		500
3	mg	0	549	0	483	0	612	0	928
	Hairs		349		298		352		528
	Index		636		617		575		569
4	mg	0	489	0	662	0	529	0	841
	Hairs		250		296		236		370
	Index		511		447		446		440
5	mg	0	569	1	026	0	709	0	937
	Hairs		323		463		300		346
	Index		568		451		423		369
6	mg	1	252	1	113	1	041	0	739
	Hairs		578		476		428		270
	Index		462		428		411		365
7	mg	0	186	0	427	0	537	0	766
	Hairs		71		152		191		184
	Index		382		356		356		240

hairs per mg of dried leaf) and their averages. Table II indicates some notable characteristics of each of the samples analyzed. Quality of drug was derived after determining volatile oil content, foreign organic matter, and filth contained in each lot (3, 4). Also in Table II are microscopical measurements (in microns) of the basal cells. Each number represents an average of twenty determinations on as many trichomes.

DISCUSSION AND CONCLUSION

As can be noted in Tables I and II, the poorer samples have a lower hair count when compared to choicer lots of drug. If a new lot of sage were ground and four samples taken, the mean hair count for these four samples would be 500 ± 102 (5). If the count were 398 or lower, or 602 or higher, a 95% certainty would then exist that the new lot differs from the above standard mean.

Hair counts obtained by the manner described can be utilized in making percentage determi-

TABLE II

Sample No	Quality	Amount Purchased	Entire or Powdered Leaves	Basal Cell Width	Dimensions, μ Length
1	Good	2 lbs.	Powdered	11.4	12.9
2	Good	2 lbs	Entire	10.9	13.2
3	Good	4 oz.	Powdered	11.2	14.0
4	Good	2 lbs	Entire	11.1	10.5
5	Poor	1 lb.	Entire	11.3	13.3
6	Poor	4 oz.	Entire	11.0	12.0
7	Poor	2 lbs	Entire	17.5	21.0

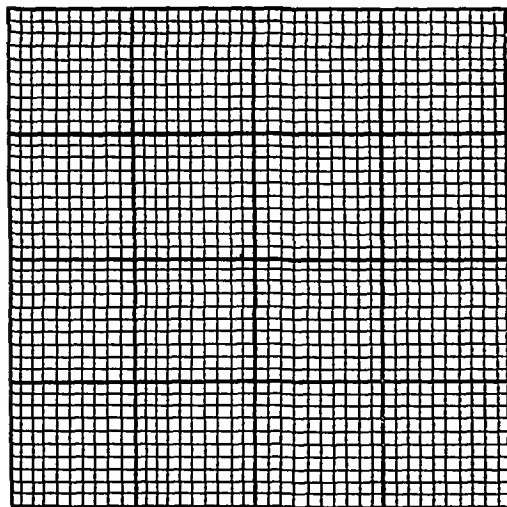


Fig. 2—Lucite plate ruling.

nations of sage leaf in various spiced foods, as will be shown in a subsequent paper, by simply extracting the vegetable material and analyzing for hair content. It is necessary, however, to take into consideration that a considerable variation may occur from lot to lot due to fertility of soil, climate, length of growing season, etc. The hair count thus obtained will indicate the content of sage leaf by comparing with suitable charts of hair count on pure samples of commercial sage.

REFERENCES

- (1) Silverman, H. I., and Dunn, M. S., *Am. J. Pharm.*, 128, 225 (1956).
- (2) Schneider, A., "Microanalysis of Powdered Vegetable Drugs," 2nd ed., Blakiston Philadelphia, 1921, p. 155.
- (3) "Pharmacopoeia of the United States of America," 15th Rev., Mack Printing Company, Easton, Pa.
- (4) Association of Official Agricultural Chemists, "Official Methods of Analysis," 8th ed., A. O. A. C., Washington, D. C., 1955, p. 1003.
- (5) Brownlee, K. A., "Industrial Statistics," Chemical Publishing Co., Brooklyn, N. Y., 1949, p. 51.

A Study of the Root of *Morinda citrifolia* Linné I*

By HEBER W. YOUNGKEN, Sr.†

Morinda citrifolia Linné, a shrub or small tree of the *Rubiaceae* family native to southern Asia, the Philippines, and islands of the South Pacific, possesses roots, extracts of which have been stated to possess hypotensive and anticongestive properties, but very scanty work has been recorded on their pharmacognosy. In this paper the physical characteristics, histology, and description of the powdered root are discussed. Among the diagnostic anatomical characteristics of this drug are the following: the presence of raphides of calcium oxalate in raphide sacs of the cortex and phloem; a banded secondary xylem; vessels with closely set, circular bordered pits; abundant starch-bearing wood parenchyma; vascular rays from 1- to 5-cells in width; stone cells in both cortex and phellem; starch grains, simple and 2- to 3-compound. The presence of an endotrophic fungus with septated, branched hyphae was observed in all tissues of the roots obtained from Luzon.

THIS STUDY WAS PROMPTED by the report in the literature by Dr. Dang Van Ho of Viet Nam (1) who stated that the total extract of the root of *Morinda citrifolia* L. was effective in 81 per cent of his 58 patients suffering from hypertension without having observed a single instance of intolerance and, also, that the drug was shown to possess anticongestive properties in the treatment of hemorrhoids, nasal congestion, and cerebral hemorrhage.

Most of the prior investigations on this drug have been concerned with chemical constituents and microchemic tests and with the root bark and wood, the former having been used in India for many years as a source of a dye. In 1848, Anderson (2) isolated from the root bark of *M. citrifolia* a crystalline substance possessing the formula $C_{25}H_{30}O_{15}$ and named it morindin. Later, Oesterle (3) isolated from this bark a substance, which he found to be trioxymethylanthraquinone monomethyl ether to which he ascribed the formula $(C_{16}H_{18}O_5)$. Oesterle and Tisza (4) isolated two other anthraquinone substances which they named morindadiol $(C_{15}H_{10}O_4)$, m. p. 244° ; and soranjidiol $(C_{15}H_{10}O_4)$, m. p. 270° ; and also a wax $(C_{18}H_{28}O)$ in white needles, m. p. 124.5° . In 1908, Tunmann (5) published a brief description of the wood and root and, by means of microchemic tests, found morindin in the medullary rays, soranjidiol in the phloem parenchyma, and morindadiol in the sieve tubes.

Since *M. citrifolia* roots have been imported for screening into the U. S. A. and as there is a possibility of the drug being used here in the future, this investigation was undertaken in order to provide sufficient pharmacognostical characterization for its identification.

MATERIALS AND METHODS

The materials used in this study included certified herbarium specimens of leaf and flowering and leaf and fruiting branches of *M. citrifolia* L. Sources of materials were as follows: one, with a portion of the root system attached, collected by Prayob Sinclermisiri in Bangkok, Thailand, in 1956 and furnished the writer by Dr. K. Savatabandahu of the Section of Systematic Botany of Bangkok Experimental Station, Bangkok; another with roots and a photograph of the plant from which they were collected in Calcutta, India, from Dr. S. C. Datta of the Central Drugs Laboratory, Calcutta; nearly five pounds of roots of different thicknesses of *M. citrifolia* L., donated by Riker Laboratories; a herbarium sheet of leaf and flowering branch and root, gathered by Dr. Ed. Quisumbing in the Philippines; and roots of *M. citrifolia* L., gathered in Luzon and certified by Dr. R. E. Woodson, Jr., Senior Taxonomist at the Missouri Botanical Garden.

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All of the root materials were first examined organoleptically. After recording their physical characteristics, samples of them were prepared for examination by the usual pharmacognostic methods discussed by the writer in previous papers in THIS JOURNAL. Sections were cut by the sliding microtome, and by freehand sectioning, and separately examined in temporary mounts in chloral solution, water, KOH solution, and other reagents discussed in this paper. The slender ends of roots were imbedded in paraffin and sectioned on a rotary microtome. Numerous temporary mounts and stained permanent mounts of transverse, radial longitudinal, and tangential longitudinal sections of root specimens were made and examined microscopically. Schulze's maceration process was used in the technique of separating the lignified elements for study. Numerous measurements of tissue elements were made and recorded.

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The Plant.—*Morinda citrifolia* L., commonly known as Indian Mulberry and/or Bankoro, is a glabrous shrub or tree of the *Rubiaceae*, attaining a height of 3–10 M. It occurs in India, Indo-China, Malaya, Thailand, Phillippines, and Polynesia. From studies of herbarium specimens, the leaves are broadly elliptic to elliptic-obovate; 10–31 cm. in length; acute, acuminate, or obtuse, less often emarginate, at the summit; cuneiform at the base; the margin nearly entire or undulate; coriaceous in texture; the lamina with from 6 to 7 areolate, ascending nerves which alternate on each side of the midrib. The petiole measures up to 12 mm. in length. The stipules are interpetiolar, suborbiculate, and 2- to 3-lobed at their summit. The stem branches are quadrangular and jointed. The inflorescence consists of solitary axillary capitula of ovate to spheroidal shape, bearing tubular white flowers. The flowers show a gamosepalous calyx with a truncate limb; a 5-lobed, tubular white corolla; 5 stamens with short, hairy filaments; and a pistil with a 2-celled ovary. The fruit is a fleshy sorosis of greenish-white to white or yellow color; up to 4 cm. in length in the material examined; mamellate on the surface, and consists of many coalescent drupes.



Fig. 1.—*Morinda citrifolia* L. leaf and flowering branch of plant growing in India. To right, below, a segment of root of same. $\frac{1}{2} \times$

Physical Characteristics of Root.—The roots examined ranged from elongated conical roots with many thin rootlets toward the distal end to broader segments of thicker roots, the latter constituting most of the drug imported into this country. The commercial drug occurs in cylindrical to subcylindrical segments, some being curved, 2–15 cm. in length and up to 5 cm. in width, occasionally up to 9 cm. in width at the crown. Externally it is reddish brown, pale yellow where abraded, marked by irregular longitudinal wrinkles, some of the thicker pieces exhibiting transverse lenticles, cir-

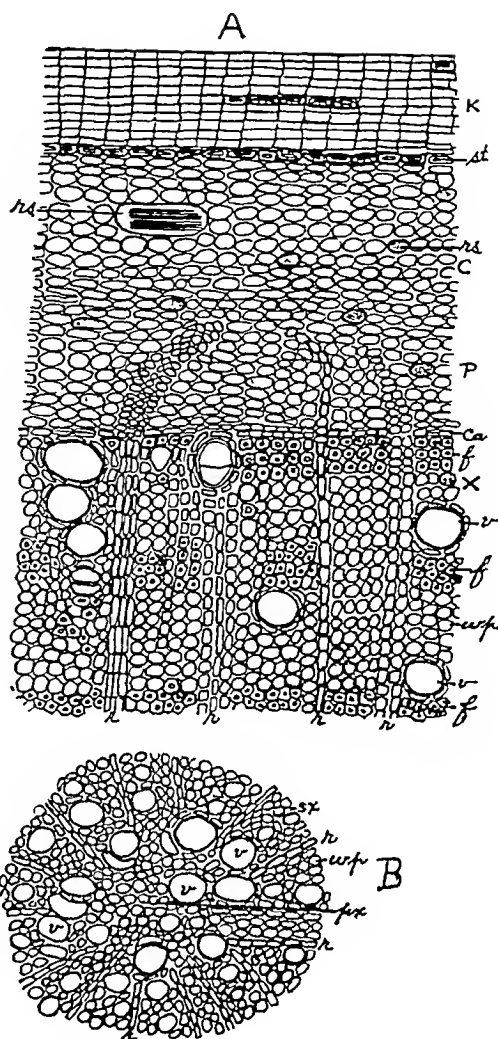


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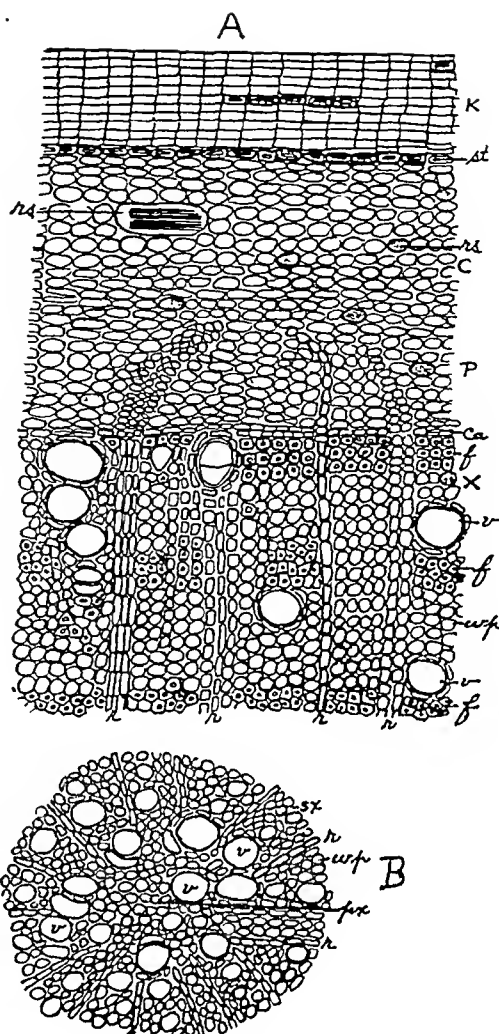


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(secondary cortex); a narrow phloem, devoid of lignified elements; a cambium and a narrow zone of secondary xylem; and a relatively broad, central primary xylem occupying about four-fifths the diameter of the rootlet. In a cross section 13 mm. in diameter, the bark occupied 2.5 mm.; the wood, 11.5 mm. The cork zone here was up to 18 layers of cells deep. The cells were rectangular to square, in regular radial arrangement, and with suberized walls. The cork cells were from 19μ to 66μ in length and from 11μ to 20μ in depth. Some of the layers showed ovate to oblong stone cells with nearly uniformly thickened lignified walls having distinct pore canals. They occurred in the outer, middle, and inner cork zones and frequently extended a considerable length through the layers, but isolated ones also occurred. All of the sections of thicker roots up to 5 cm. possessed this stone cork. Raphide saes were numerous, scattered throughout the phloem and cortex. The phloem was traversed by phloem rays usually up to 3 cells wide, but occasionally up to 4, rarely 5, cells wide. The phloem ray cells took on a deep red color with KOH solution. The secondary xylem consisted of numerous wood wedges separated by xylem rays of straight to curved character whose cells walls were pitted and whose contents consisted of starch and anthraquinones. The vessels were mostly isolated in the wedges, but also occurred in groups of 2 to 4; usually arranged radially. A number of them exhibited tylosis. In the Luzon roots, many were plugged with fungal hyphae and gummy lignin. A large primary xylem in the center of the section showed pitted wood parenchyma in which vessels, mostly isolated, were scattered. The secondary xylem was invariably banded in this, as well as in broader sections of older root parts. The bands consisted of groups of wood fibers with lignified walls arranged in concentric circles between which occurred wood parenchyma through which vessels and tracheids coursed. In radial longitudinal and tangential longitudinal sections the raphide saes, up to 120μ long and up to 38μ wide, were arranged singly and in vertical rows in the cortex and phloem. They were filled with acicular crystals of calcium oxalate, the latter up to 180μ in length. In tangential sections, the sieve tubes showed laterally placed sieve plates. The xylem rays were mostly 1- to 3-cells wide, occasionally 1- to 4-cells wide. The vessels possessed closely set bordered pits and oblique ends with large circular openings in the perforation plates. They measured up to 418μ in length and up to 152μ , very rarely up to 200μ , in width. The tracheids were mostly shorter, but like the vessels possessed closely set, bordered pits. Those measured were up to 240μ in length and up to 102μ in width. The wood parenchyma was abundant, its cells being up to 162μ in length and 42μ in width. It contained starch grains and anthraquinones. The wood fibers were straight to curved, some of them showed irregular lobes or teeth toward one or both ends. They were nonseptate. Their ends were mostly pointed and their walls lignified, showing oblique pits. These fibers were up to 1380μ in length and up to 38μ in width.

Sections of the roots from Luzon showed the presence of branched, septated hyphae of an endotrophic fungus which had penetrated into cells of all of the tissues, even occurring within the lumina of

vessels, tracheids, and wood fibers. The fungus was probably mycorrhizal in origin.

Powdered *M. citrifolia* Root.—This was brown to reddish—or purplish brown—and possessed a slightly sweet taste. Under the microscope it showed: numerous fragments of wood fibers crossed by cells of xylem rays—the fibers with irregularly thickened, lignified walls and with narrow to broad lumina; numerous starch grains—single to 2- to 3-compound, the individual grains spheroidal, plano-convex, angular convex, pyriform and ovoid, the larger grains with a distinct ecentric hilum, the grains up to 26μ in diameter and showing in the larger grains polarization crosses when examined under polarized light; scattered fragments of vessels and tracheids with closely set circular bordered pits; fragments of vascular rays with pitted walls; scattered fragments of cork whose cells are polygonal to rectangular with suberized walls; scattered cork stone cells with nearly uniformly thickened, lignified walls; numerous acicular crystals of calcium oxalate; and many fragments of elongate starch-bearing wood parenchyma. When mounted in 2% KOH solution, the tissue fragments took on a pink to red color, due to the solution of anthraquinones within the parenchyma cells and rays flowing out into the mount.

Work is in progress on a second paper in which the results of chemical and pharmacological studies on this root will be reported.

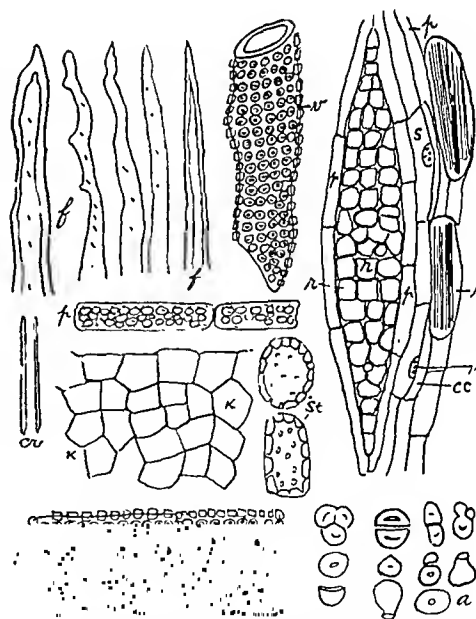


Fig. 3.—Histological elements occurring in longitudinal sections and in powdered *Morinda citrifolia* L. root. *f*—ends of wood fibers; *v*—vessel with closely set bordered pits; *r*—vascular ray of phloem region with adjacent tissues as seen in longitudinal tangential section; *p*—phloem parenchyma; *st*—sieve tube; *cc*—companion cell; *rs*—raphide sac with acicular crystals of calcium oxalate; *cr*—acicular crystals; *p*—starch parenchyma; *K*—cork; *St*—stone cells from cork region; *tr*—tracheids with bordered pits; *a*—starch grains (magnified).

SUMMARY

1 A brief history is given of the drug and its new clinical use in Viet Nam as a hypotensive and anticongestant.

2 A description is given of the plant materials and methods used in this study of the root of *Morinda citrifolia* which came from Thailand, India, and the Philippines

3 A description is given of *Morinda citrifolia* L. based mostly on herbarium materials used by the author

4 The physical characteristics of the root and the commercial drug are described

5 The histology of the root of *M. citrifolia* is discussed. Among the microscopical features are the occurrence of stone cells in the cork, the absence of lignified elements in the cortex and phloem, a

banded xylem composed of concentrically arranged groups of wood fibers, the sieve plates on the lateral walls of the sieve tubes, and vessels and tracheids with closely set circular bordered pits on their walls

6. The occurrence of anthraquinones in the parenchyma and vascular rays as reported by Tunmann (4) is verified.

7 The powdered root of *M. citrifolia* is described

REFERENCES

- (1) Van Ho, D, *Presse méd*, 63, 1478(1955)
- (2) Anderson, Th, *Ann Chem u Pharm*, Liebig's 71, 216(1849)
- (3) Oesterle, O A, and Tisza, E, *Arch Pharm*, 245, 287(1907).
- (4) Oesterle, O A, and Tisza, E, *Arch Pharm*, 246, 150 (1908)
- (5) Tunmann, O, *Pharm Zentralhalle*, 49, 1013 (1908)
- (6) Lecomte, M H, "Flora Generale Indo China", "Rubiaceae," J Pitard, Masson et Cie, Vol 3, p 423, Paris 1924

Modification of Rates of Gastrointestinal Absorption of Drugs I. Amines*

By CHESTER J. CAVALLITO and ROSEMARY JEWELL

Tannates of several therapeutic amines have been prepared and shown to be reproducible compositions containing one part of amine for each galloylgallic acid moiety of tannic acid. Dialysis experiments were conducted to determine the influence of a number of factors on the rate of release of diffusible amine from the relatively insoluble complex. In the presence of aqueous solutions of electrolytes amines are slowly released from their tannates and the rate increases with the $[H^+]$. Polygalacturonic acid can reduce the rate of release of free amine from the tannate, particularly at lower pH. The application of these principles to the preparation of oral repository forms of therapeutic amines is discussed.

ALMOST ANY THERAPEUTICALLY active compound has some period of action during which there exists the optimum condition of desired response with least embarrassment of undesirable symptoms possible for that particular drug. If it were possible to provide a drug in such form that its rates of absorption and loss were suitably balanced to a more sustained level of optimum action, material improvement in therapy would result. The goal of much of the effort expended in the search for new drugs has been to find compounds which more effectively possess this characteristic. While success of such efforts at improvement is underscored by the rapid rate of obsolescence of products in the pharmaceutical industry, it is recognized that many of the older drugs for which no adequate replacement has been found and some of the newer ones

would perform more satisfactorily if it were possible only to extend their period of optimum response. In the last several years a number of new approaches to this problem has been evident in the area of oral therapy. Although the oral route of administration is by far the most convenient, it is also the most difficult to control because of the variations in gastrointestinal environment among different individuals, with the same individual at different times and in different portions of the gastrointestinal tract.

Many medicinal agents are organic amines or their salts. If one desires a long response, the dosage of amine required is usually so high that for some period of time undesirable symptoms of over-dosage may result. Among parenteral preparations, the use of salts or complexes of low solubility has made possible more uniform and prolonged responses to medication. During the past few years, several oral preparations have

* Received June 29, 1957 from The Research Laboratories, Irwin, Neisler & Co., Decatur, Ill

been introduced which utilize relatively insoluble forms of a drug under conditions which also provide more uniform and prolonged responses. In our laboratories the complexing agent which has been found to be particularly useful for amines is tannic acid. The favorable results which have been obtained clinically (1) with the use of amphetamine tannate¹ in providing oral repository amphetamine has led to the preparation and study of other amine tannates.

Tannates of many organic bases are of low aqueous solubility but in the gastrointestinal tract they will release the base for absorption. Although the release is not contingent upon any specific pH, the rate is greater in environments of low pH than under neutral or slightly alkaline conditions. Some practical implications of this are discussed later.

Tannates of a variety of types of amine therapeutic agents have been made in these laboratories from tannic acid N F (2). A surprising characteristic of such preparations has been their relative uniformity of composition. Gallotannic acid may be idealized structurally as the pentadigallic ester of glucose, although the N F preparation is undoubtedly a mixture of closely related depside glucosides. In the tannate complexes the resulting products have a ratio of one amine for each digallic moiety of the tannic acid.

Since the purpose of preparing oral repository forms of drugs is to provide, usually in the human, a longer period of optimum response, the evaluation of ultimate significance depends upon the performance of the preparation in practice. Recently there have been several reports describing *in vitro* procedures for measuring relative rates of release of amines from complexes with anionic polymers (3, 4). Such studies of the properties of these products may be of some value in gaining further understanding of their action and in providing approaches for improvements, but it is appreciated that release rate measurements obtained from arbitrary *in vitro* experiments can be only suggestive of behavior in humans.

EXPERIMENTAL

Preparation of Amine Tannates—The preparative procedures vary slightly with the solubility of the amine, but a convenient method was found to be the following. The amine base was dissolved in a small volume of an alcohol (methyl, ethyl or propyl alcohols) and for each molar equivalent of base there was added 374 Gm of tannic acid N F in a solution of the alcohol. This represents a 10% excess of one fifth the molecular weight (1701)

of pentadigallic glucose, $C_6H_5O_{46}$. The mixture was diluted with an excess of ice water to complete precipitation of the tannate. The resulting precipitate was filtered off, washed thoroughly with ice water, and dried in a vacuum oven to yield an amorphous solid varying from nearly white to yellow in color.

The free amine base may be used or may be liberated by addition of the calculated quantity of sodium or potassium hydroxide to alcohol or aqueous alcohol solution of the amine salt. Phenethylamines, such as amphetamine, form tannates which are quite soluble in methanol and which are prepared in concentrated solution prior to dilution with ice water. Alkaloids, such as atropine and morphine, on the other hand, form less soluble tannates in alcohol which precipitate when prepared in concentrated solutions, although incompletely without dilution with water. Antihistamines of the dimethylammonioalkylpyridine type react quite cleanly, presumably with the more basic amino group to form monobasic complexes. With the less soluble tannates, such as those of morphine, codeine, atropine, etc., a nearly quantitative recovery of base is obtained.

TABLE I—COMPOSITION OF TANNATES

Base	—% Basic N in Compositions—			
	Tannate, found	Calcd for Compositions, Base Tannic Acid Ratios	4:1	5:1
Amphetamine	2.8	2.50	2.95	3.35
Morphine	2.21	1.97	2.24	2.46
Atropine	2.16	1.96	2.22	2.45
Propietyrid amine	4.74	4.21	4.82	5.31

The therapeutic base content of the tannate complex was determined by titration of the product in glacial acetic acid with standardized perchloric acid solution. The basic nitrogen contents of representative types of amine tannates are shown in Table I. There also are shown the theoretical basic nitrogen contents of ratios of 4, 5 and 6 to 1, respectively, of base to tannic acid (assumed $C_{76}H_{52}O_{46}$). It is seen that the 5 to 1 ratio best represents the compositions.

The 5 to 1 ratio of amine to tannic acid appears to provide the least soluble composition. Preparations formed in which the amine ratios were smaller than this yielded more water-soluble products.

Release of Amines from Tannates—The general procedure used for measuring release of amine from its insoluble tannate was as follows. A weighed amount of finely powdered amine tannate (or other salt for comparison) equivalent to 50 mg of amine base was added to 100 ml of the aqueous test medium in a Type SS Visking tubing² (presoaked in water for thirty minutes) which had been tied at one end to form an open end $1\frac{7}{8}$ inches in diameter, 6 inches long. This was immersed in 225 ml of aqueous test medium contained in a 400 ml beaker.

¹ Synthane brand of amphetamine, Irwin Neisler & Co.

² Seamless, regenerated cellulose dialysis tubing, average pore radius 21 \AA , Visking Corporation, Chicago, Ill.

so that the liquid levels of the beaker and the sac contents were the same. The contents were stirred vigorously and continuously. Exploratory experiments were first carried out both with the beaker contents undergoing continuous replacement with fresh aqueous test medium (rate of 50 to 100 ml. per minute) and with the original solution retained for establishment of an equilibrium. At regular intervals, a 5-ml. sample was withdrawn from the dialysate or sac contents for analysis to determine the extent of dialysis of water-solubilized amine. Propenpyridamine maleate was used to determine the difference in diffusion by these two procedures. After 1, 2, 3, 4 and 6 hours' dialysis, the amounts of amine dialyzed with continuous replacement of dialysate were approximately 15% greater than in experiments with dialysate unchanged. Because of ease of manipulation, and since the data are primarily of comparative value, the procedure was selected in which the dialysate was not replaced.

The results obtained from the dialysis experiments are listed in Table II. The ease of quantitative

with morphine in which the quantitative assay procedure was as follows. A 5-ml. aliquot was transferred to a small separatory funnel to which was added 2 ml. of 5% aqueous sodium bicarbonate solution. The procedure previously described for propenpyridamine was then followed excepting that instead of chloroform, a 4 to 1 (by volume) chloroform-*n*-butyl alcohol mixture was used. The absorbance was read at 285 μ within a preferred concentration range of 2–10 mg. per 100 ml.

Among the factors considered as possibly influencing release of an amine from its tannate complex were pH and electrolytes. Potassium phosphate buffers (0.01 molar) of pH 3 to 9 were used. The rate of diffusion of propenpyridamine as its maleate salt was found to be essentially the same in distilled water as in the buffers. Propenpyridamine tannate suspended in distilled water showed very little release of amine from solution and dialysis. The buffer electrolytes had an appreciable solubilizing effect which increased with lower pH values. Experiments were conducted at 25°; a few run at

TABLE II.—DIALYSIS OF AMINES

Compound	Medium	Retardant	% Dialyzed of Equilibrium Distribution ^a						
			Hours Dialyzed ^b						
			1	2	3	4	6	12	24
Propenpyridamine Maleate	Water ^b	24	33	45	55	68
Propenpyridamine Tannate	Water	<1	..	9	14
Propenpyridamine Tannate	Buffer, pH 3	19	28	37	49	64	83	95
Propenpyridamine Tannate	Buffer, pH 5	12	16	21	30	47	65	81
Propenpyridamine Tannate	Buffer, pH 7	8	10	14	18	23	35	51
Propenpyridamine Tannate	Buffer, pH 9	<1	7	9	13	19	30	49
Propenpyridamine Tannate	Buffer, pH 3	Polygalacturonic Acid	12	19	22	31	41	51	73
Propenpyridamine Tannate	Buffer, pH 5	Polygalacturonic Acid	8	15	18	24	32	45	62
Propenpyridamine Tannate	Buffer, pH 7	Polygalacturonic Acid	..	12	15	19	26	36	47
Propenpyridamine Tannate	Buffer, pH 9	Polygalacturonic Acid	<1	9	13	18	23	31	40
Propenpyridamine Maleate	Buffer, pH 3	Polygalacturonic Acid	20	29	37	46	56
Tannic Acid ^c	Buffers pH 3-7	7	13	19	26	37
Morphine Sulfate	Buffers pH 3-9	24	36	45	54	67	..	100
Morphine Tannate	Buffer, pH 3	..	16	27	38	44	58	80	90
Morphine Tannate	Buffer, pH 7	..	9	19	24	30	43	62	77

^a Per cent in dialysate of that concentration of amine which would be present if all of that placed in the dialysis sac had become equally distributed throughout the total 325 ml. of fluid.

^b Distilled water; 0.01 molar potassium phosphate buffers of pH 3 to 9 have no significant influence on these results.

^c Tannic acid diffusion; no amine.

analysis led to the use of the antihistaminic agent, propenpyridamine, in exploring the influence of several variables on availability of drug from tannate complex under the *in vitro* conditions. The 5-ml. aliquot removed for assay was transferred to a small separatory funnel to which was added 1 ml. of 2% aqueous sodium hydroxide solution. This solution was extracted three times, each with 5 ml. of chloroform. The filtered, combined chloroform extracts were shaken out with 5 ml. of 0.05 *N* aqueous HCl. The absorbance (with suitable dilution, if necessary, to 1–5 mg. per 100 ml.) was measured at 260 μ and the concentration determined from a standard curve prepared for propenpyridamine.

A few comparative experiments were carried out

38° showed only a slight increase in rate of amine release.

Tannic acid itself can diffuse through the Visking membrane. Its rate of diffusion, however, is appreciably slower than that of the dissolved amine. After solution in an electrolyte medium, the tannate molecule would not be expected to offer much hindrance to escape of the amine. In practice it has been found advantageous to include pectic or polygalacturonic acids in oral compositions containing amine tannates. This polyanionic natural polymer provides an additional means for influencing the rate of release of free amine. (Mechanical as well as physical-chemical influences probably play a part.) The polygalacturonic acid was found to be

³ Exchange Lemon Products Company, Corona, Calif.

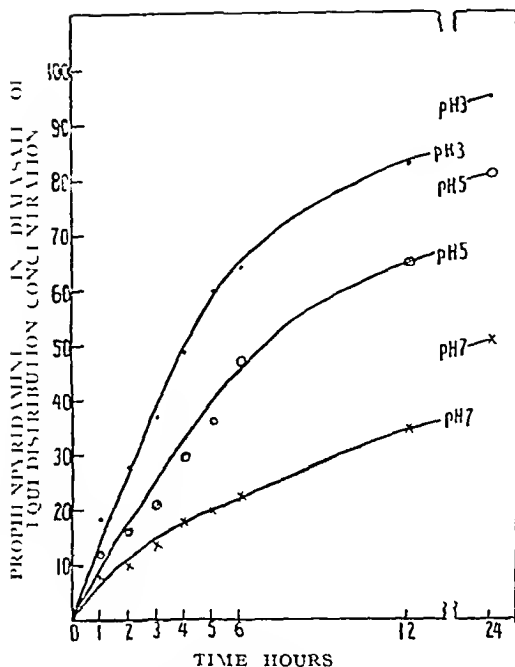


Fig 1—Prophenpyridamine Tannate Influence of pH Per cent dialysed of that concentration of prophenpyridamine which would be present in the dialysate if all of the prophenpyridamine tannate placed in the dialysis sac had become equally distributed

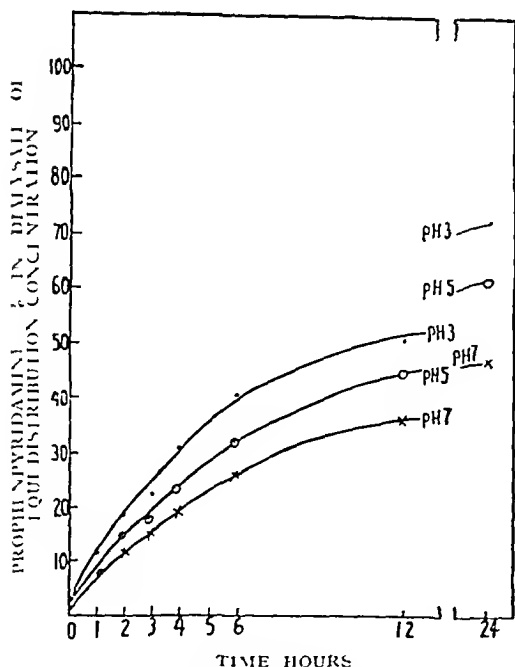


Fig 2—Prophenpyridamine Tannate + Polygalacturonic Acid Influence of pH Per cent dialysed of that concentration of prophenpyridamine which would be present in the dialysate if all of the prophenpyridamine tannate placed in the dialysis sac had become equally distributed

nearly free of material diffusible through the membrane. Controls were run with the polygalacturonic acid and a correction made for its UV absorption. The influence of a 1% suspension on amine diffusion was measured after adjusting the suspension to several pH values with 0.1 N HCl or NaOH. A significant retardant action is evident at pH 3 and 5 with practically no effect at 7 and 9. The polygalacturonic acid is thus a more effective retardant of the amine at lower pH values, tannates are inherently less soluble in neutral or slightly alkaline environments.

A soluble morphine salt was shown to have about the same rate of diffusion as the soluble antihistamine. The influence of pH on morphine tannate was analogous to that shown by the prophenpyridamine tannate.

DISCUSSION

It has been well known for many years that alkaloids form water-insoluble precipitates with tannins. There have been relatively few investigations of these compositions and these usually with rather crude preparations. The tannates derived from therapeutic amines and tannic acid N F have shown surprisingly uniform compositions. Recently, they have been applied in the preparation of oral repository forms of therapeutic amines. Although individual tannates vary considerably in solubility, amines of therapeutic interest generally are sufficiently large that their tannates are relatively insoluble in water. It has been shown that the presence of electrolytes and lowering of pH increases the rate of release of amine from tannate. Addition of polygalacturonic acid to the media can reduce appreciably the rate of dialyses of amine, particularly in environments of lower pH in which the tannates would usually be more soluble. An increase in $[H^+]$ would increase the concentration of carboxyl groups in the polycarboxylic retardant which would be available for salt formation and bonding with the amine. In practice, polygalacturonic acid is used concomitantly with the tannate to help provide the rate of initial release of amine found to be indicated by responses in humans. With the tannates, all components ultimately become individually dissolved and the therapeutic amine becomes totally available.

Sympathomimetic amines bound to insoluble cation exchange resins (5,6) also have been used as oral repository preparations. The mechanism of bonding of amines to insoluble cation exchange resins is apparently complex (7) and other variables are involved in addition to some of those influencing rate of release of amines from tannates.

REFERENCES

- (1) Garrett T A *Clin Med* 3, 1185 (1946)
- (2) U S Dispensatory p 1379 24th Edition 1955 Lipincott Philadelphia
- (3) Kennon I and Higuchi T *This Journal* 45, 157 (1956) 46, 21 (1957)
- (4) Chaudhry N C and Saunders I *J Pharm and Pharmacol* 8, 975 (1956)
- (5) Larsen D H U S Patent 2,498,687 (1950)
- (6) Freed S C Keeling J W and Hays I I *Am Pract Dir Treatment* 7, 19 (1956)
- (7) Saunders I and Srivastava R *J Chem Soc* 1950, 2915

Modification of Rates of Gastrointestinal Absorption of Drugs II. Quaternary Ammonium Salts*

By CHESTER J. CAVALLITO and THOMAS B. O'DELL

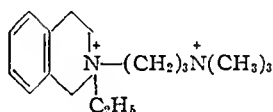
From physical-chemical and physiological bases, several factors had been rationalized as possibly influencing oral absorption of quaternaries. These originally were evaluated in animals and later with *in vitro* studies. It has been found that in animals, lowering of intestinal pH, the administration of certain sterol acids or the addition of biologically inactive quaternaries all improve the oral responses to a quaternary hypotensive agent. *In vitro* studies have supported suggested explanations for some of these effects. Practical applications are currently under evaluation.

IN THE PRECEDING ARTICLE (1) there were discussed approaches to the control of rates of oral absorption of therapeutic amines in which the problem was to prevent too rapid initial absorption and to prolong the period of time during which drug action would be maintained. With quaternary ammonium salts, a significant and often limiting factor in oral therapy is their irregular and usually poor absorption from the gastrointestinal tract. Several techniques have been developed in our laboratories which, based on evidence from physical measurements and biological responses, result in improvement in oral effectiveness of quaternaries.

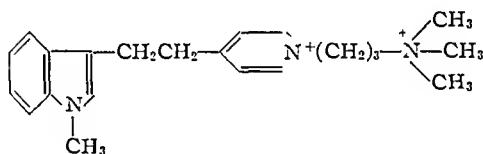
During the past several years, many types of diquaternary ammonium salts have been tested in animals in these laboratories for not only their biological activities by parenteral routes but also for their relative oral effectiveness. Ratios of oral to intravenous equipotent doses have been determined in animals and occasionally in the human. The variations have ranged from about 10 to 1 to several hundred times to 1 ratios between oral and intravenous effective doses. That these ratios are usually high has been well recognized. It is generally assumed that the ionized character of quaternaries renders many living membranes difficultly permeable to them. Presumably, anionic groups present in these membranes retain the cations by electrostatic bonding and displacement is slow by an ion exchange mechanism. Ruth Levine and co-workers (2), in an investigation of absorption and excretion rates of quaternaries in animals, have suggested a plausible hypothesis that mucins of the gastrointestinal tract act as barriers to quaternary absorption by formation of stable complexes. Irrespective of the exact location of the chemical structures barring quaternary absorption from the gut, there are several possible mechanisms for

circumventing adsorption and facilitating absorption.

The quaternaries used in these studies were two unsymmetric bisquaternary hypotensive agents, IN 292 (3a,b) and IN 391 (4a,b), which showed about 20 to 1 ratios between oral and intravenous effective dosages in humans (5) and in dogs. Anything useful in increasing permeability of the gut to quaternaries must be physiologically innocuous. One of the classes of compounds considered was that of the bile acids.



IN 292



IN 391

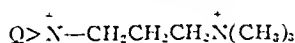
These are known to influence oral absorption of a number of substances. It was rationalized that, in solution, a salt complex formed between a diquaternary and a bile acid might be less readily subject to ion exchange and fixation of quaternary at anionic receptors in the intestinal wall. Experiments in animals demonstrated a significant favorable influence of cholic acid, for example, on oral effectiveness of IN 292. This was confirmed in humans (5) in which 150 to 200 mg of cholic acid doubled the oral effectiveness of similar quantities of IN 292. The limitation in practice was that the bile acids would produce a laxative effect which was not always desirable.

The next approach was rationalized as follows. The relative affinities of quaternaries for adsorption at sites of loss in the intestine probably are unrelated to the activities of those quaternaries

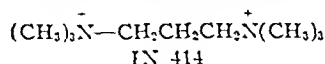
* Received June 29, 1957 from The Research Laboratories, Irwin, Neisler & Co., Decatur, Ill.

The authors are indebted to Mrs. Martha D. Napoli and Mrs. Rosemary Jewell for technical assistance and to Dr. A. P. Gray for some of the chemicals.

as hypotensive agents. It might, therefore, be possible to administer, together with the hypotensive agent, a quaternary which was physiologically inert but which could effectively compete at sites of loss in the gut, thereby resulting in greater availability for absorption of free unadsorbed therapeutic quaternary. Since the hypotensive agents were compounds of type



in which Q is a large group, an ideal potentiator might be a salt of the simple compound



which biologically is relatively inert. Experiments in animals and humans showed that this diquaternary was appreciably effective in improving responses to oral medication with IN 292 or IN 391. Concomitant intravenous administration in animals of IN 414 with the hypotensive agents showed no significant potentiation by this route. The mechanism appears to involve potentiation by competition in the gastrointestinal tract at sites of loss of the active quaternaries.

In addition to IN 414, several other closely related, biologically relatively inert, diquaternaries were tested for their ability to potentiate in dogs the response to "orally" administered IN 391. The corresponding C₂ and C₄ homologs also were effective, but less so than the C₃ derivative, IN 414. The bis-trimethylammonium analog of IN 414 similarly was not quite as effective although it did potentiate.

Still another variable considered was that of pH and its influence on relative bonding affinities of quaternaries for polyanionic receptors. It was found that with increasing hydrogen ion concentrations, appreciably less fixation of quaternary at polyanionic receptors was evident.

Oral effectiveness of IN 391 in dogs was found to be significantly increased by increasing the acidity of contents of loops of intestine.

In the experimental section there are discussed data which support the preceding conclusions and permit speculations as to implications of these studies.

EXPERIMENTAL

In vitro Diffusion Measurements.—The general procedure employed in measuring rates of diffusion of quaternary ammonium compound was essentially the same as that described in the preceding article (1). The quaternary used was IN 391 which showed sharp U.V. absorption maxima at 255 mμ and 288 mμ. Optical density-concentration standard curves for the compound permitted accurate determinations of quantities of the order of 2 to 5 mg. per 100 ml. At regular intervals, aliquots were taken of the dialysate and the IN 391 cation content was determined directly or after suitable dilution with water. The chloride salt of the quaternary was used throughout; several of the experiments which were repeated with bromide salt gave the same results. These salts in distilled water gave solutions of pH 6.0.

The data obtained are summarized in Table I. All experiments were conducted at least in duplicate. To 100 ml. of the solvent medium in the dialysis sac there was added 60 mg. of IN 391 chloride (50 mg. of IN 391 cation). The same solvent medium (225 ml.) less drug was added to the beaker on the outside of the sac (dialysate). At the time intervals indicated, 5-ml. aliquots were removed from the dialysate for analysis. Among the variables considered as possibly influencing diffusion of quaternaries through the inert, semipermeable membrane were pH and presence of electrolytes. The quaternary was equally distributed within and outside of the dialysis sac in about six hours with water as the solvent medium. The use of 0.01 M NaCl solution significantly reduced the rate of diffusion of the quaternary as did also 0.01 M potassium phosphate buffer. The greater retarding influence of the phosphate buffer than of sodium chloride is probably related to the greater concentration of ions in this buffer solution. Variations in pH between 4 and 8 had no influence on diffusion rate.

TABLE I.—IN 391 DIFFUSION

Solvent Medium	Retardant	Potentiator	pH	% Dialyzed of Equi-distribution Conc ^a					
				—Hours, Dialyzed—					
Water			6	41	58	..	88	95	98
0.01 M NaCl			6	29	..	56	68	79	82
0.01 M K Phosphate buffer			4-8	23	37	45	57	61	70
Water	Mucin		4	17	..	42	52	55	61
Water	Mucin		6	12	21	27	34	39	41
Water	Mucin		8	7	12	22	27	31	36
Water	Mucin	100 mg Citric Acid	3.5	11	27	..	46	57	68
Water	Mucin	100 mg Tartaric Acid	3.3	14	26	..	53	61	74
Water	Mucin	IN 414 (60 mg.)	6	20	31	42	47	53	58
Water	Mucin	IN 414 (90 mg.)	6	29	42	..	58	61	66
Water	Pectic Acid		3	<1	6	8	10	11	11
Water	Pectic Acid	IN 414 (90 mg.)	3	9	18	24	30	34	37

^a Per cent in dialysate of that concentration of IN 391 which would be present if all of that placed in the dialysis sac had become equally distributed throughout the total 325 ml. of fluid.

One of the structures which has been implicated as possibly retarding cation absorption is the mucin lining of the gastrointestinal tract (2). Experiments were carried out with a 1% suspension of Gastric Mucin Powder¹ in water as the solvent medium for 1N 391 in the dialysis sac. This preparation in distilled water had a pH of 6.0. The influence of pH was observed by adjusting to pH 4 with dilute HCl or to 8 with dilute NaOH. Mucin was shown to have a marked influence in retarding dialysis of the quaternary and the influence was particularly greater at higher pH values. The dialysis membrane was relatively impermeable to the mucin; however, a small amount of material slowly diffused through which showed some absorption in the U.V. analytical range. The diffusible impurities were dialyzed out of a 1% suspension of the mucin for a twenty-four hour period preceding use of the suspension in these experiments. The addition of 100 mg. of either citric or tartaric acid to the mucin medium also showed the influence of added organic acids (lower pH) on mucin binding of the quaternary.

In the preceding article, the influence of polygalacturonic (purified pectic) acid was evaluated as a retardant for amine absorption. This polycarboxylic acid appears to have a very marked bonding capacity for the quaternary.

In Figure 1, there are shown the relative influences of electrolytes, mucin and pectic acid on rates of 1N 391 diffusion; in Fig. 2, the influence of pH on mucin binding is demonstrated.

The ability of a biologically relatively inert quaternary (IN 414) to increase oral effectiveness of 1N 391 had been predicated on the assumption that the sites of loss of the therapeutic quaternary in the gastrointestinal tract would be blocked by adsorption of the biologically inactive potentiator. The influence of addition of 60 mg. or 90 mg. of 1N 414 chloride to the 1N 391-mucin or 1N 391-pectic acid combinations is evident from the table or Fig. 3. The IN 414 significantly reduces the apparent binding of the 1N 391 to the anionic polymers.

Pharmacological Activity Measurements.—The effectiveness of additives in potentiating the "oral" activity of quaternaries was determined as follows. Dogs were anesthetized with pentobarbital sodium and the blood pressure measured directly from the common carotid artery. The quaternary salts, in aqueous solution, were administered directly into the upper third of the small intestine. The procedure was then repeated (different dogs) using the quaternary in combination with the additive agent. Most experiments were repeated at least once. Insoluble acid additives were dissolved in 1.0 to 2.0 ml. of 2% aqueous sodium bicarbonate solution. Responses were evaluated on the basis of per cent fall in blood pressure from normal and duration of fall before return of blood pressure to pre-drug level or to level of maximum recovery. Results are summarized in Table II.

The influence of pH on intestinal absorption was measured by the same procedure in dogs. 1N 391 was administered in 10 ml. of 0.1 molar potassium phosphate buffers of pH 4.0 and 8.4 by injection into the small intestine at a point just below the distal

end of the pancreas. Tying off a six-inch loop of intestine at the area of injection enhanced the re-

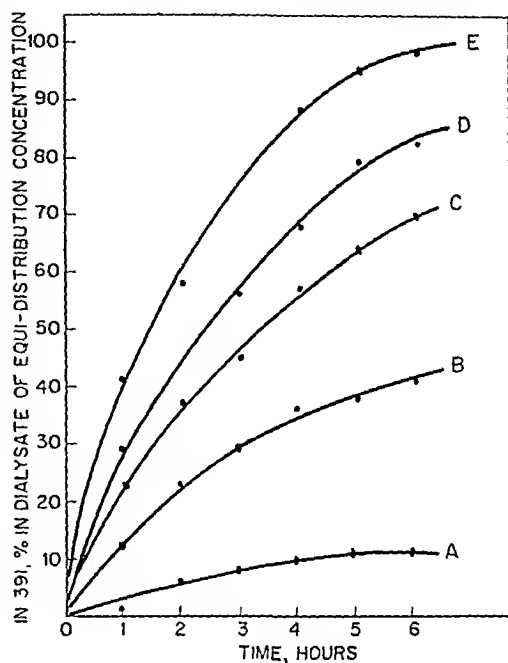


Fig. 1—Dialysis of 1N 391 from: (A) 1% pectic acid suspension in water, (B) 1% mucin suspension in water, (C) 0.01 M phosphate buffer, (D) 0.01 M sodium chloride solution, (E) water (distilled) (Table I).

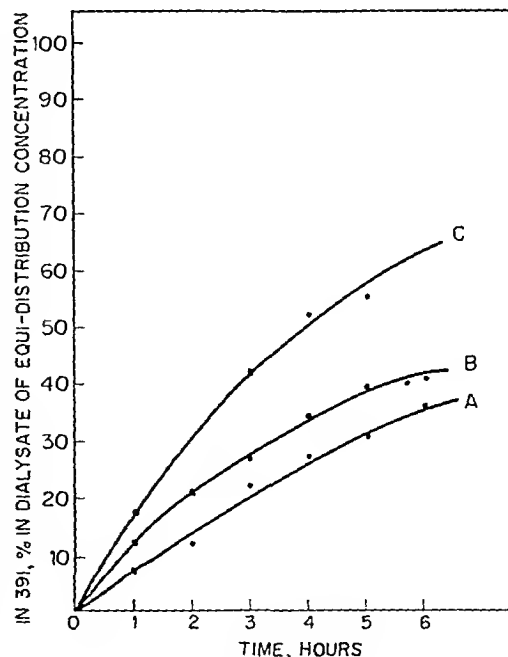


Fig. 2—Dialysis of 1N 391 from 1% mucin suspension in water at pH (A) 8, (B) 6, (C) 4 (Table I).

¹ Gastric Mucin (N.N.R., 1957, p. 386).

responses to some extent as might be expected. Results are outlined in Table III.

DISCUSSION

The erratic and often poor absorption of quaternaries by the oral route may be related to a number of gastrointestinal environmental variables. The mucin lining could well be a major barrier to their absorption from the gastrointestinal tract. The barrier effect would be expected to increase and absorption of quaternary become poorer as the environment became less acid. This might result in a more rapid absorption of quaternaries in the upper part of the gastrointestinal tract with poorer absorption later. The influence of pH on mucin binding might explain why the major part of the oral absorption of a poorly absorbed quaternary appears to occur fairly quickly (2). As the intestinal contents become less acid, quaternary binding by polyanionic food components (such as peptic acids) as well as the mucins could become more significant. The improved oral absorption of quaternaries by fasting individuals (6) might result from not only less loss of quaternary by binding to food components and dilution but possibly also from less neutralization of secreted acid. One might speculate that quaternaries which reduce gastric acid secretion may indirectly curtail absorption of later doses and lead to apparent tolerance. Reserpine has been reported to increase gastric acid secretion (7) although this is uncertain (8). Drugs which increase gastrointestinal acidity might produce some potentiation of quaternaries by improving their absorption.

The magnitude of the influence of other ions on binding of quaternaries to mucins or to other parts of the gastrointestinal wall is difficult to assess. Living membranes usually are selectively permeable to ions and different electrolytes might vary considerably in their influence on absorption of quaternaries.

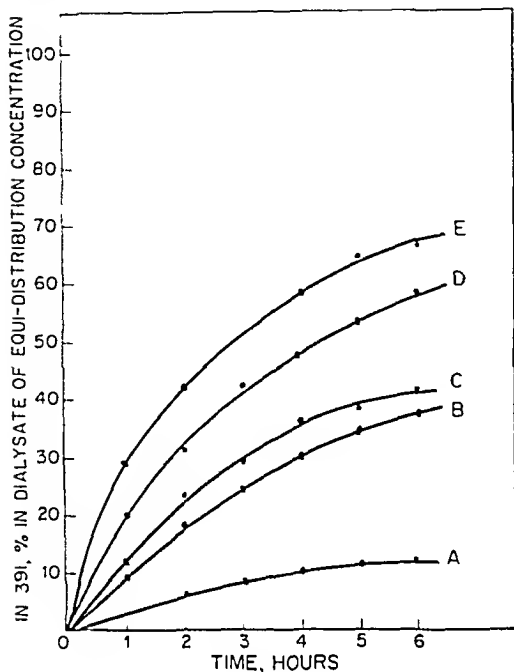


Fig 3—Dialysis of IN 391 from (A) 1% pectic acid suspension in water, (B) as in (A) but with IN 414 added, (C) 1% mucin suspension in water, (D) as in (C) but with 60 mg IN 414, (E) as in (C) but with 90 mg IN 414 (Table I).

TABLE II—INFLUENCE OF ADDITIVES ON RESPONSES TO QUATERNARY AMMONIUM HYPOTENSIVE AGENTS ADMINISTERED INTO THE INTESTINE OF DOGS

Hypotensive Quaternary	Dose mg/Kg	Additive	Dose mg/Kg	Blood Press % Fall	Duration, Hours ^a
Hexamethonium	5			45	1
Hexamethonium	10			50	2.5
Hexamethonium	5	Cholic Acid	10	50	4.5
Hexamethonium		Cholic Acid	10	0	0
IN 292	2			15	1
IN 292	4			20	2.5
IN 292	6			25	3
IN 292	4	NaHCO ₃	2	30	3
IN 292	2	Cholic Acid	10	35	>3.5
IN 292	4	Cholic Acid	5	35	>5
IN 292	4	Cholic Acid	10	60	>4
IN 292	4	Dehydrocholic Acid	10	50-70	>4
IN 292	4	Oleic Acid	10	20	3
IN 292	4	Sorbitan Monostearate	10	25	2.5
IN 292		IN 414	2	0	0
IN 292	2	IN 414	1	65	4.5
IN 292	4	IN 414	0.5	60	>2
IN 292	4	IN 414	1	65	>4
IN 292	4	IN 414	2	65	>3
IN 391	0.35			20	3
IN 391	0.5			30	>1
IN 391	1			50	>1
IN 391	0.5	Cholic Acid	10	55	>3
IN 391	0.5	IN 414	0.5	50	>3.5

^a Durations greater than three to four hours cannot be reliably measured in anesthetized dogs.

TABLE II.—INFLUENCE OF pH ON RESPONSES TO HYPOTENSIVE QUATERNARY IN 391 ADMINISTERED INTO THE INTESTINE OF DOGS

Duration, Hours	Blood pH	mg/Kg IN 391 Press	% Fall	35	4
3	Open	0.35	20	35	4
>2.5	Open	0.35	30	0	0
0	Open	0.35	0	0	0
0	Open	0.5	0	45	4
0	Loop	0.5	0	0	0
8.4	Loop	0.5	0.5	0	0
8.4	Loop	1.0	0.5	35	4

The mutual influences of ions on their intestinal absorption is apparently complex (9). Experiments also have demonstrated that two (particularly duration) to the two quaternary hypotensive agents. The surface-active, lipophilic oleic acid and sorbitan monostearate have practically no influence on the responses to the quaternaries. Although cholic acid lowers blood pressure when injected parenterally, it and the related conjugated bile acids do not appear to enter the circulation when administered orally and have no influence on blood pressure (10, 11). Control experiments in the presence of oral cholic acid alone. The mechanism of this influence on quaternaries is not clearly understood. The steroid acids might increase permeability of the intestinal wall; they might have a mild mucolytic action thereby reducing the barrier effects of intestinal mucus; they may form salts with quaternaries and provide enough anion molecular bulk to reduce the frequency of ion exchange. The last mechanism is not unreasonable and might permit more facile passage of the quaternary salt of the steroid acid across a lipophilic living membrane. In any event, the implication is that individual vari-

REFERENCES

(1) Cavallito, C. J., and Jewell, R., *This Journal*, 47, 166(1958).
(2) Levine, R. M., Blair, M. R., and Clark, B. B., *J. Pharmacol. Exptl. Therop.*, 114, 78(1955).
(3) Gray, A. P., Archer, W. L., Schlieper, D. C., Spinner, E. B., and Cavallito, C. J., *J. Am. Chem. Soc.*, 77, 3356(1955). (b) O'Dell, T. B., Luna, C., and Napoli, M. D., *J. Pharmacol. Exptl. Therop.*, 114, 317(1955).
(4) Gray, A. P., Archer, W. L., Spinner, E. B., and Cavallito, C. J., *J. Am. Chem. Soc.*, 79, 3805(1957). (b) *Therop.*, 120, 438(1957).
(5) Fries, B. D., personal communication.
(6) Harrington, M., *Chem. Soc.*, 12, 185(1953).
(7) Clunton, M. L., and Schneider, E. M., *Gastroenterology*, 29, 877(1955).
(8) Rider, J. A., *Proc. Soc. Exptl. Biol. Med.*, 90, 636(1955).
(9) Ingraham, R. C., and Vischer, M. B., *Am. J. Physiol.*, 101, 121, 771(1933).
(10) Carrere, G., and Gerard, B., *Bull. Acad. Med.*, 107, 443(1932).
(11) Le Gac, P., *Bull. Soc. Sci. Bretagne*, 23, 45(1918).
(12) *Chem. Abstr.*, 44, 2654(1950).

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By W. MOROZOWICH and F. W. BOPE†

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The 8-nitrothecophyllinates of aniline, aminepyrine, diphenhydramine, atropine, and cocaine were prepared. The presence of two melting points with the aniline salt indicates its unsaturation. Attempts to prepare the 8-nitrothecophyllinate of antipyrine were unsuccessful.

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REFERENCES

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- (5) Freis, E. D., personal communication
- (6) Harrington, M., *Clin. Sci.*, 12, 185(1953)
- (7) Clark, M. L., and Schneider, E. M., *Gastroenterology*, 29, 877(1955)
- (8) Rider, J. A., *Proc. Soc. Exptl. Biol. Med.*, 90, 636(1955)
- (9) Ingraham, R. C., and Visscher, M. B., *Am. J. Physiol.*, 121, 771(1938)
- (10) Carriere, G., and Gerard, E., *Bull. Acad. Med.*, 107, 443(1932), *Chem. Abstr.*, 27, 4591(1933)
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(4) (a) Gray, A. P., Archer, W. L., Spinner, B. E., and Cavallito, C. J., *J. Am. Chem. Soc.*, 79, 3805(1957). (b) O'Dell, T. B., and Napoli, M. D., *J. Pharmacol. Exptl. Therap.*, 120, 338(1957).
(5) Preis, E. D., personal communication.
(6) Harrington, M., and Schneider, E. M., *Gastroenterology*, 29, 877(1955).
(7) Clark, M. L., and Schneider, E. M., *J. Biol. Med.*, 90, 636(1955).
(8) Rider, J. A., *Proc. Soc. Exptl. Biol. Med.*, 90, 636(1955).
(9) Ingraham, R. C., and Vischer, M. B., *Am. J. Physiol.*, 121, 771(1938).
(10) Carriere, C., and Gerard, E., *Bull. Acad. Med.*, 107, 413(1932). *Chem. Abstr.*, 27, 4591(1933).
(11) Le Gac, P., *Bull. Soc. Sci. Bretagne*, 23, 45(1918).
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- (10) Carriere, G., and Gerard, E., *Bull. Acad. Med.*, 107, 443(1932), *Chem. Abstr.* 27, 4591(1933)
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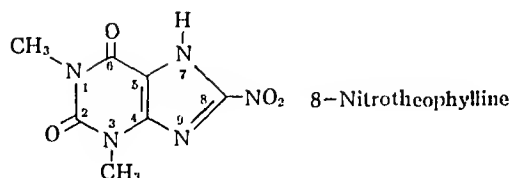
bases (1) and the antihistaminics (2), have been reported. The 8-chlorotheophylline salt of diphenhydramine was the first of these compounds to be made. Drowsiness, one of the main untoward reactions of antihistaminics, was found to be diminished (3) in these compounds by virtue of the CNS stimulating property of the theophylline moiety.

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Increased acidity should result in more stable salts with less chance of precipitation of the free xanthine derivative in the stomach.

Dusel, *et al.* (4), have prepared the choline salts of theophylline, 8-chlorotheophylline, and 8-bromotheophylline. The oral LD_{50} 's of these compounds were found to range between 500-850 mg./Kg. The LD_{50} of the choline salt of 8-nitrotheophylline was nonlethal at 5,000 mg./Kg.

The purpose of this work was to prepare the 8-nitrotheophyllinates of some antihistaminic and analgesic compounds and to attempt synthesis of the aniline and the aminopyrine salts which could not be prepared as the 8-chlorotheophyllinates (1).

concentrated to 30 ml. by distillation, then cooled to -5° . The yellow crystals obtained were recrystallized twice from 99% isopropyl alcohol, then dried over P_2O_5 at 90° and 3 mm. for three hours. The m. p. was $191-196^{\circ}$. Microscopically the crystals were clustered together in the form of yellow rosettes. Titration of a 0.0405 Gm. sample required 1.001 equivalents of standard NaOH, indicating that the salt consists of a 1:1 ratio of 8-nitrotheophylline to aminopyrine. Infrared spectrographs were obtained for the starting materials and characteristic absorptions were identified in the final compound.

The following compounds were similarly prepared: aniline-, diphenhydramine-, cocaine-, and atropine 8-nitrotheophyllinate. Crystal structure, C, H analysis and melting points of the compounds are reported in Table I. All of the compounds were found to be 1:1 ratios of base to 8-nitrotheophylline.

DISCUSSION

The m. p. of the aniline 8-nitrotheophyllinate taken conventionally on a Fisher-Johns apparatus was $279-283^{\circ}$, approximately the same as that of 8-nitrotheophylline. However, when the compound was dropped onto a preheated melting point block

TABLE I. 8-NITROTHEOPHYLLINATES

Compound	M p. ^a	Crystal Nature	Formula	C, H (Calcd.)	C, H (Found) ^b	Yield, %
Aminopyrine 8-Nitrotheophyllinate	191-196	Yellow rosettes	$C_{21}H_{21}N_5O_2$	52.63, 5.30	52.57, 5.40	45
Diphenhydramine 8-Nitrotheophyllinate	197-198	Yellow rods	$C_{21}H_{21}N_2O_2$	59.99, 5.87	60.20, 5.82	63
Cocaine 8-Nitrotheophyllinate	103-111	Yellow rods	$C_{21}H_{21}N_4O_2$	54.51, 5.31	54.42, 5.54	48
Atropine 8-Nitrotheophyllinate	202-206	Yellow rosettes	$C_{21}H_{29}N_4O_2$	56.62, 5.87	56.10, 5.85	43
Aniline ^c 8-Nitrotheophyllinate	155 ^d	Yellow parallelograms and some elongated hexagons	$C_{11}H_{11}N_2O_2$	49.66, 4.43	50.86, 5.74	45

^a Melting points were taken on a Fisher-Johns melting point block.

^b Carbon-hydrogen analyses were carried out by Schwarzkopf Laboratories, New York, N. Y.

^c Composition in doubt due to discrepancies in C, H analyses and to crystal nature. See Discussion.

^d When dropped onto a hot melting point block, it melts above 155° , then solidifies, and melts again at $279-283^{\circ}$, which is approximately the same as for 8-nitrotheophylline.

EXPERIMENTAL

8-Nitrotheophylline was prepared by nitrating theophylline in glacial acetic acid according to the method of Dusel, *et al.* (4). Potentiometric titration of 8-nitrotheophylline with standard sodium hydroxide solution established the pK_a at 3.58. The m. p. of 8-nitrotheophylline was $282-283^{\circ}$ with decomposition.

Aminopyrine 8-Nitrotheophyllinate.—A sample of 0.450 Gm. (0.0200 mole) of 8-nitrotheophylline was refluxed with 0.502 Gm. (0.0216 mole) of aminopyrine in 150 ml. of 99% isopropyl alcohol. After refluxing for one hour the clear yellow solution was

it melted when the temperature was above 155° , then solidified, and melted again at $279-283^{\circ}$. This indicates that the compound is quite unstable and probably reverts to the starting materials when above 155° .

REFERENCES

- (1) Lamb, D. J., and Bope, F. W., *THIS JOURNAL*, 45, 178 (1956).
- (2) Corsic, J. W., U. S. Pat. 2,534,235-2,534,247.
- (3) Gay, L. N., and Carliner, P. E., *Science*, 109, 359 (1949).
- (4) Dusel, B. F., Berman, H., and Schater, R. J., *THIS JOURNAL*, 43, 619 (1951).

A Study of the Effect of Certain Pharmaceutical Materials on Color Stability*

By ROY KURAMOTO, LEON LACHMAN, and JACK COOPER

The influence of several pharmaceutical materials on the fading of the certified color, FD&C Blue No. 2, has been investigated. It has been found that sugars such as dextrose, lactose, and sucrose increase the rate of fading, whereas sugar alcohols such as mannitol and sorbitol do not appreciably affect the rate. However, if trace amounts of strong reducing catalysts remain in the sugar alcohol after being crystallized, they significantly affect color stability. In general, antioxidants such as hydroquinone, *p*-hydroxypropiophenone and hydroquinone monomethyl ether were found not to appreciably retard the fading due to reducing sugars. In the case of NDGA, however, the rate of fading due to dextrose is substantially reduced. It appears that the rate of fading of the above color is catalyzed in the presence of reducing compounds.

ALTHOUGH COLOR FADING of pharmaceutical preparations appears to be a common occurrence, no previous reports on the mechanism of the reaction seems to have been published in the literature. It has been a common practice in the pharmaceutical industry to discard the use of a specific color if it is found to be unstable in a given formulation. This was done without determining the agent or agents which were responsible for this reaction. This approach is now becoming more difficult to utilize since the number of certified dyes has been diminishing due to recent FDA investigations and subsequent rulings. Therefore, it appeared to be desirable to investigate the actual mechanism of the reaction in order to possibly retard the rate of fading.

The certified dye, FD&C Blue No. 2, an indigo sulfonate, was utilized in this investigation. This dye was chosen since it was found to be very unstable in the presence of many pharmaceutical ingredients or when subjected to different environmental conditions. It was decided to study the rate of fading of this dye in solution rather than in formulations such as tablets, suspensions, etc.; since this form is more amenable to stability investigation and chemical analysis. In addition, the instability of this dye would lend itself to a thorough study within a reasonable length of time.

From the chemical structure of the dye and actual experience with color stability in product formulations, it appeared that the fading was due to an oxidation-reduction reaction as well as to a dependency on light intensity. However, in this study no attempt was made to isolate the contribution of the light intensity factor to the overall reaction.

In order to substantiate the above reasoning, the effect on color stability caused by various reducing and nonreducing sugars, sugar alcohols, antioxidants, and other compounds was evaluated. Experimental data indicate that sugars such as dextrose, lactose, and sucrose increase the rate of fading; whereas sugar alcohols such as sorbitol and mannitol do not appreciably affect the rate. However, if trace amounts of strong reducing catalysts remain in the sugar alcohol after being crystallized, it significantly affects color stability. In general, antioxidants such as hydroquinone, *p*-hydroxypropiophenone and hydroquinone monomethyl ether were found not to appreciably retard the fading due to reducing sugars. In the case of NDGA, however, the rate of fading due to dextrose is substantially reduced.

PREVIOUS STUDIES

A search of the literature reveals that although there are numerous reports on the fading of vat dyes in the textile industry, there is a conspicuous lack of reports on the mechanism of color fading in pharmaceutical formulations. One of the few investigations on color stability in pharmaceutical preparations has been reported on by Garrett and Carper (1). They recently reported on the color stability of a liquid multi-sulfa preparation by kinetic investigation of the thermally accelerated degradation of the color. They did not attempt to determine the mechanism of the reaction or the ingredients which were responsible for the fading. Their main purpose was to predict the shelf color stability of this formulation in a brief period of three and one-half weeks, which they appeared to have accomplished successfully.

The effect of light intensity on textile color stability has been reported on by Taylor and Pracejus (2) and others (3-8). In general, most investigators report that color fading in textiles is dependent on light intensity. Desai and Vaidya (5) indicated that dyes such as Brilliant Green, Malarhite Green, Crystal Violet, Rosaniline, and others were stabilized against sunlight to varying degrees by the addition

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of copper sulfate. Results of Taylor and Pracejus (2) indicate that the fading of colored textiles by daylight is principally due to radiant energy in the visible spectrum.

The food industry, in many respects, has similar problems with color stability as do the pharmaceutical companies. For example, an interesting study by Pruthi and Lal (9) on the color of citrus juices has shown that sweetening agents present in the product tended to accelerate the color changes. This is in agreement with the observations found with pharmaceutical formulations containing similar type sweetening agents.

There have been numerous publications on the oxidation-reduction reactions of various dyes. It appears that the color fading of indigo is due to the formation of a leuco compound preceded by an unstable intermediate (10-12). In addition, it has been shown that metallic ions catalyze the oxidation or reduction of indigo type colors (13-15).

It is apparent from the above cited references that dyes are readily affected by reducing and oxidizing agents as well as by light intensity. Since many pharmaceutical formulations contain colorants as well as various ingredients which possess reducing or oxidizing properties, it appeared desirable to investigate the color stability of these preparations. This approach appeared essential in order to determine the mechanism of the reaction from which one can postulate a possible method of retarding the rate of fading.

EXPERIMENTAL

Materials Used. Sucrose U. S. P., dextrose U. S. P., lactose U. S. P., mannitol N. F., sorbitol (Atlas Powder Co.), sorbitol solution N. F. (Sorbitol, Atlas Powder Co.), hydroquinone (Merek & Co.), nordihydroguaiaretic acid (NDGA, K & K Laboratories, Long Island City, N. Y.), hydroquinone monomethyl ether (Eastman Chemical Products, Inc.), *p*-hydroxypropiophenone (Eastman Kodak Co.), FD&C Blue No. 2 (Calco Chemical Division, Lot 68652), and phosphate buffer solution, pH 6.6.

Equipment. Beckman spectrophotometer model DU, Beckman recording spectrophotometer model DK-1, and Beckman pH meter model G.

Procedure.—Aqueous buffer solutions (pH 6.6) containing 0.15% dye, 0.15% dye + 5% sugar or sugar alcohol, 0.15% dye + 5% dextrose + 0.1% chemical additives, and 0.15% dye + 0.1% chemical additives were prepared and investigated for their color stability. These samples were stored at room temperature and exposed to ordinary room illumination. At designated time intervals, aliquot samples were removed and assayed spectrophotometrically for residual color at 610 m μ where $E_{1\%}^{1\text{cm}} = 248$.

RESULTS AND DISCUSSION

The stability of FD&C Blue No. 2 in a buffered aqueous solution was investigated in the presence of several materials commonly found in pharmaceutical preparations. These studies were buffered at pH 6.6 in order to maintain a constant hydrogen ion concentration as well as to approximate the pH of a 0.15% aqueous solution of the dye.

The influence of various sugars on the rate of fading of the dye is presented in Table I and Figs.

TABLE I.—INFLUENCE OF SUGARS ON THE RATE OF FADING OF FD&C BLUE NO. 2 AT pH 6.6 AND AT ROOM TEMPERATURE

Sugar	Rate $\times 10^3$ (Days ⁻¹)
Dextrose	19.60
Sucrose	9.20
Lactose	9.78

1 and 2. It can readily be seen from Fig. 1 that dextrose substantially increases the rate of fading after a period of induction. This "lag time" is probably due to the formation of a semiquinone intermediate which is very unstable. A straight line plot after the initial lag time indicates an apparent first order reaction with respect to the dye concentration.

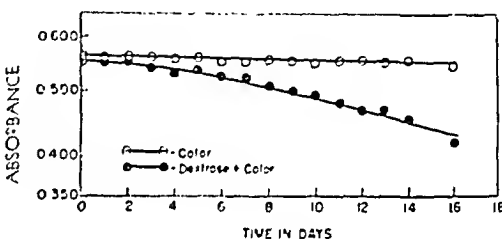


Fig. 1.—A plot showing the influence of dextrose on the rate of fading of FD&C Blue No. 2 where the upper line represents the rate of fading of the dye in the absence of dextrose.

From Fig. 2 it is apparent that lactose and sucrose affect the rate of fading in a manner similar to dextrose. However, it appears that the monosaccharide has a greater influence on the color instability than the disaccharides. Although sucrose is a nonreducing sugar, it still causes fading, but to a lesser degree than lactose. This is essentially due to the hydrolysis of the sucrose to form dextrose and fructose both of which have reducing groups.

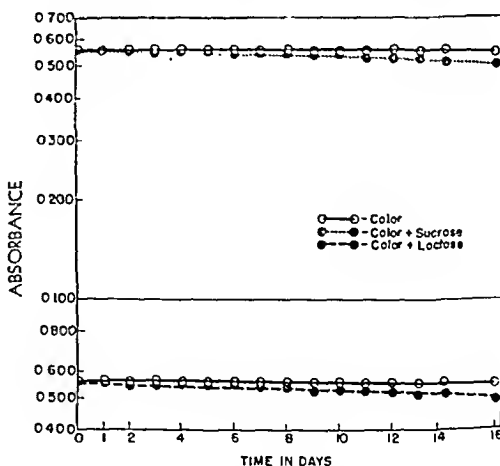


Fig. 2.—A plot comparing the effect of sucrose and lactose on the rate of color fading.

From the data presented in Table II and Fig. 3, it seems that sorbitol and mannitol do not appreciably affect the rate. However, it can be seen from the curve of sorbitol Lot 634 that some batches of sorbitol significantly affect the rate of color disappearance. This may be due to the presence of trace amounts of strong reducing catalysts which were not completely removed during the purification stage. Although the actual method of preparation and purification of this particular batch of sorbitol is not readily available, two methods of preparation have been reported in the literature (16, 17) which utilize strong reducing catalysts such as nickel and iron. The curve representing sorbitol Lot 634 does not show an induction period and this can be attributed to the more rapid formation of the semiquinone which is subsequently converted to the leuco form. This strongly indicates that such materials should be adequately tested by the Quality Control Division before being used in pharmaceutical formulations containing colorants.

TABLE II. INFLUENCE OF SUGAR ALCOHOLS ON THE RATE OF FADING OF FD&C BLUE NO. 2 AT PH 6.6 AND AT ROOM TEMPERATURE

Sugar Alcohols	Rate $\times 10^3$ (Days ⁻¹)
Sorbitol (Lot 1709)	2.04
Sorbitol Solution N. F.	7.36
Sorbitol (Lot 634)	27.60
Mannitol	7.36

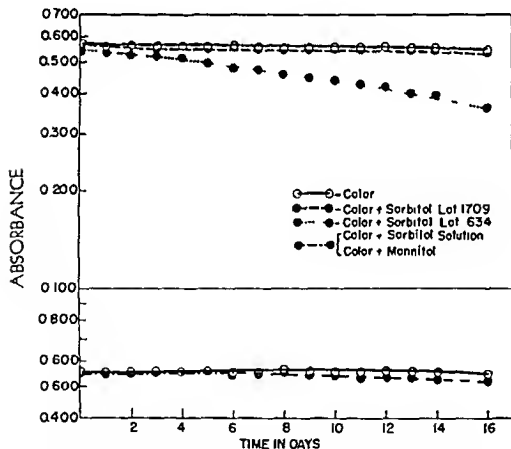


Fig. 3—The influence of various sugar alcohols on the rate of fading of FD&C Blue No. 2

In order to determine the effect of some antioxidants and related chemicals on the rate of color fading, various solutions containing these agents were investigated. The information obtained from these studies is presented in Table III and Figs. 4-7. It is apparent from data in Table III and Fig. 4 that the rate of color fading in the presence of dextrose and hydroquinone monomethyl ether is approximately the sum of the rates of each compound. However, in the case of hydroquinone, as shown in Fig. 5 and Table III, the rate of fading appears to be greater than the additive effect of

TABLE III.—INFLUENCE OF ANTIOXIDANTS AND RELATED CHEMICALS ON THE RATE OF FADING OF FD&C BLUE NO. 2 AT PH 6.6 AND AT ROOM TEMPERATURE

Additive	Rate $\times 10^3$ (Days ⁻¹)
Hydroquinone monomethyl ether	6.90
Hydroquinone monomethyl ether + dextrose	28.50
Hydroquinone	7.36
Hydroquinone + dextrose	33.40
<i>p</i> -Hydroxypropionophenone	0.00
<i>p</i> -Hydroxypropionophenone + dextrose	19.60
NDGA	9.20
NDGA + dextrose	9.20

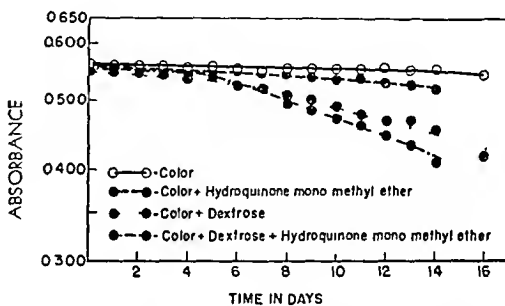


Fig. 4—The effect of hydroquinone monomethyl ether on the rate of color fading due to dextrose

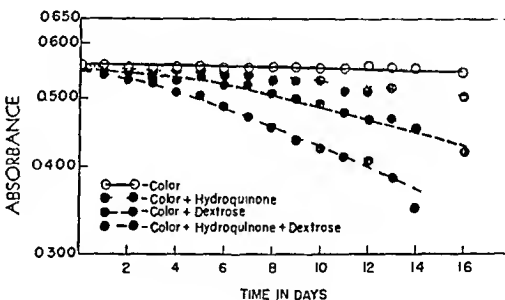


Fig. 5—A graph indicating the influence of hydroquinone and dextrose on the rate of color fading

dextrose plus hydroquinone. The rate is essentially equal to the sum of the rate due to dextrose plus twice the rate due to hydroquinone. This may be attributed to the two easily oxidizable groups present in the hydroquinone molecule. Whereas, with *p*-hydroxypropionophenone (Fig. 6), it can readily be seen from Table III that this compound did not contribute to the overall rate. This may be explained by the presence of a relatively strong electron attracting group on the molecule in the *para* position. In contrast to the above additives, the presence of NDGA in the color solutions retards the effect due to dextrose. It can be seen from Fig. 7 that the rate of fading is essentially due to the effect of NDGA and the dextrose does not appear to contribute to the overall effect. This may be explained

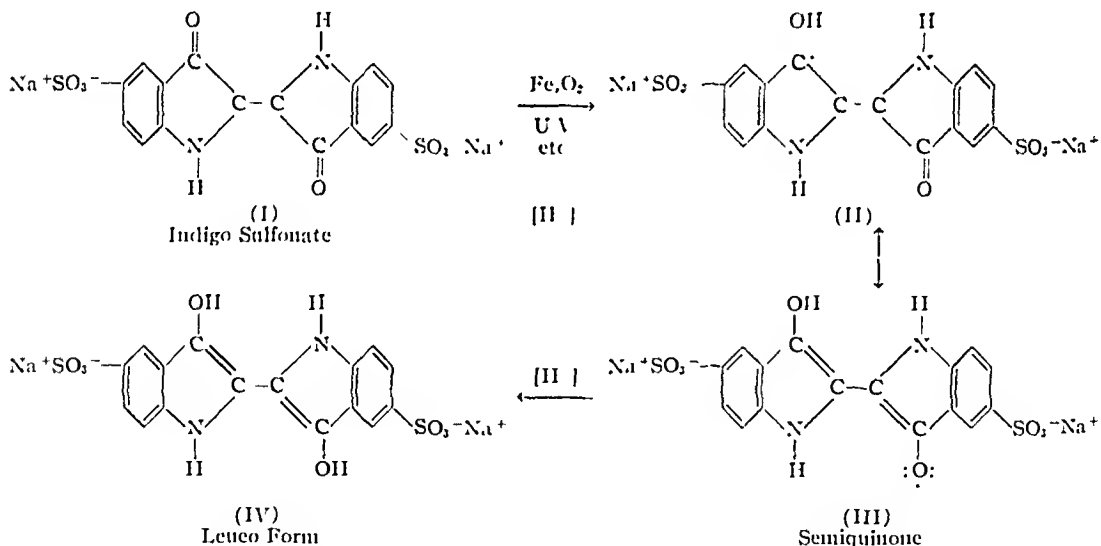
by the relative ease of oxidation of NDGA as compared to that of dextrose

It can be seen from Figs. 8 and 9 that there is a significant change in the absorption spectrum both in the visible and UV regions. Figure 8 illustrates the changes in intensity of the color due to the extent of reduction to the colorless leuco form. However, in Fig. 9, the curves indicate a change in the structure of the dye molecule upon reduction. The absorption maximum at 285 m μ appears to decrease as the carbonyl group is reduced. This is further substantiated by the formation of a new maximum at 235 m μ which is probably due to the reduced form of the carbonyl group.

Mechanism.—A search of the literature did not appear to reveal a comprehensive mechanism for the reduction of indigo dyes. However, Appleton and Geake (12) indicate that indigo undergoes reduction through the formation of an intermediate compound. From their results, they further conclude that this reduction mechanism involves a two-electron process. This appears to be in agreement with the work of Moran and Stonehill (8) who have shown that the oxidation-reduction of anthraquinoid vat dyes proceeds by a free radical mechanism.

From the above studies and the data obtained in this investigation, the following possible mechanism is proposed

Proposed Mechanism of Reaction



Propagation of Reaction

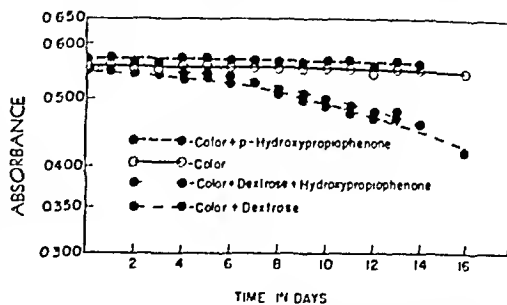


Fig. 6—A plot showing the insignificant effect of *p*-hydroxypropiophenone on the rate of fading due to dextrose

The semiquinone (II & III) gives only two of the several possible contributing forms to the resonance hybrid. However, it would appear that III is probably the most stable electronic configuration of this group.

The induction period observed in this study is probably due to the build-up of the semiquinone which is subsequently reduced to the leuco form.

Derivation of the Rate Equation.—Assuming the above mechanism is representative of the reaction

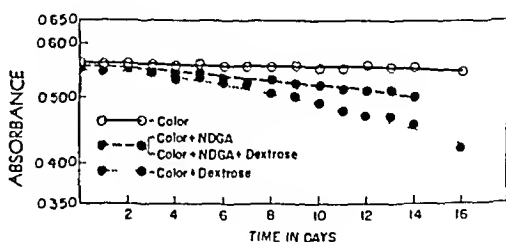


Fig. 7—The retarding effect of nordihydroguaiaric acid on the rate of fading of FD&C Blue No. 2 due to dextrose.

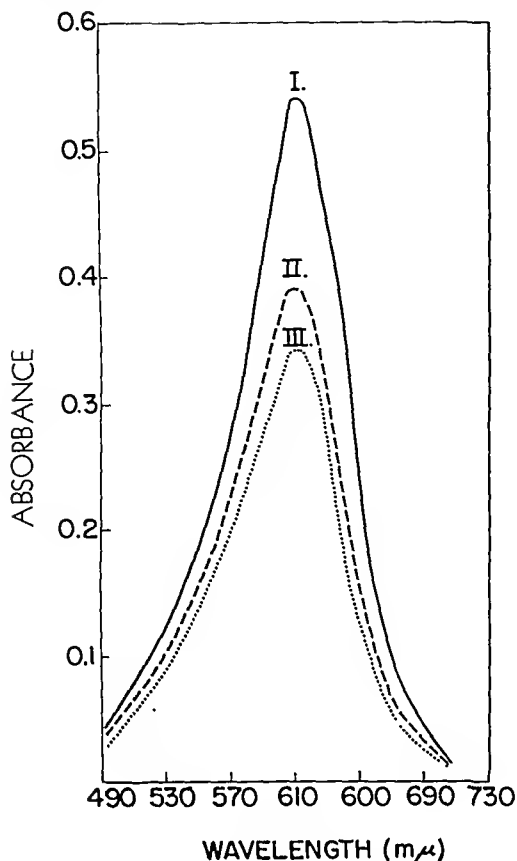
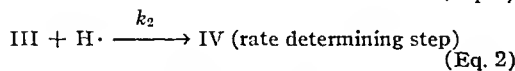
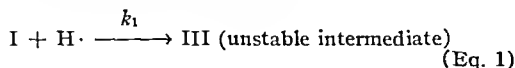


Fig. 8.—A plot of the visible absorption spectra of FD&C Blue No. 2 indicating the different degrees of color fading in the presence of reducing substances. Curve I represents the initial absorption maximum of the color.

and utilizing the experimental data, the following rate equation can be derived:



$$\frac{d(IV)}{dt} = k_2 (III) (H\cdot) \quad (\text{Eq. 3})$$

$$\frac{d(III)}{dt} = k_1 (I) (H\cdot) - k_2 (III) (H\cdot) \quad (\text{Eq. 4})$$

Since III is assumed to be an unstable intermediate, the "steady state method" may be employed to compare the postulated mechanism with the empirical data. This method is applicable only to those cases in which the intermediates are unstable and, therefore, their concentrations are at all times much smaller than those of the reactants. For such cases, it is reasonable to postulate that after a time, which is very short relative to the half-time of the reaction, a steady state is attained and during the steady state the rate of change of the concentrations of the intermediates is negligibly small.

On the basis of this assumption, one may set the time derivatives of the concentrations of each of the intermediates equal to zero.

If

$$\frac{d(III)}{dt} = 0, k_1 (I) (H\cdot) = k_2 (III) (H\cdot) \quad (\text{Eq. 5})$$

Therefore:

$$\frac{d(IV)}{dt} = k_1 (I) (H\cdot) = -\frac{d(I)}{dt} \quad (\text{Eq. 6})$$

Equation 6 is representative of the overall reaction which is given by the mechanism above.

Since the concentration of the reducing agent is so much greater than the dye concentration, it can

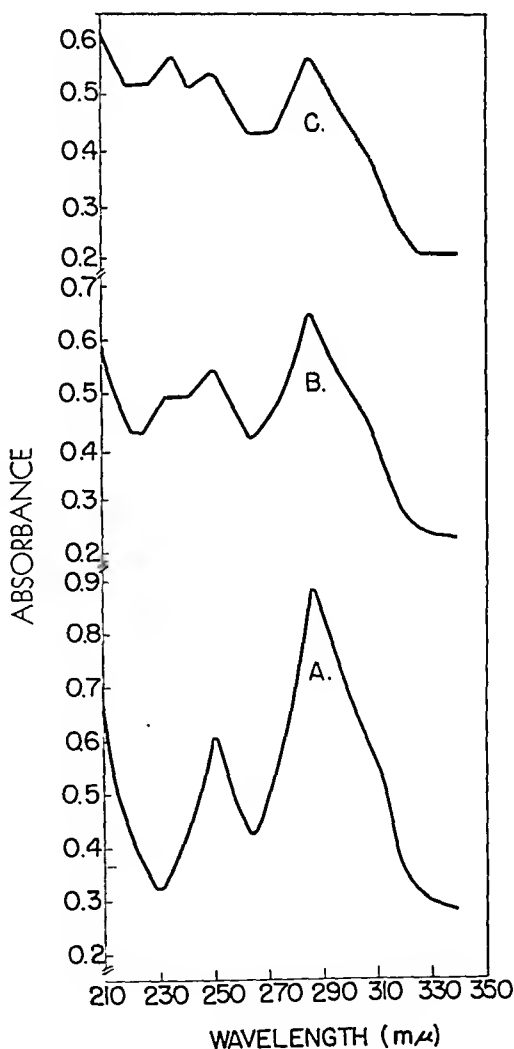


Fig. 9.—A plot of the ultraviolet absorption spectra of FD&C Blue No. 2 indicating a change in the spectra due to the extent of formation of the colorless leuco form. Curve A represents the initial absorption spectrum of the dye.

be assumed that the hydrogen free radical term in Eq. 6 is constant

Then:

$$-d(I)/dt = k_1(I) \quad (\text{Eq. 7})$$

This shows that the assumed mechanism is consistent with the observed first order kinetics.

SUMMARY AND CONCLUSIONS

The effect of several pharmaceutical materials on the rate of fading of FD&C Blue No. 2 has been investigated. From the experimental data obtained and the postulated mechanism, the following conclusions can be drawn

1. The fading of FD&C Blue No. 2 appears to be readily potentiated by reducing agents.

2. In general, the addition of antioxidants does not appreciably retard the rate of fading of the color in the presence of dextrose

3. Of all the antioxidants investigated in this study, only NDGA appeared to substantially decrease the rate of fading due to dextrose

4. The presence of trace impurities in commonly used pharmaceutical ingredients may cause

color fading.

5. A possible mechanism for the reaction has been proposed which is based upon a two-electron change.

6. The "steady state method" was employed in the derivation of an apparent first-order rate equation for the overall reaction which appears to be in agreement with the experimental data.

REFERENCES

- (1) Garrett, E. R., and Carper, R. F., *THIS JOURNAL*, **44**, 515(1955)
- (2) Taylor, A. H., and Praeger, W. C., *Illum. Eng.*, **45**, No. 3, 110(1950)
- (3) Morton, T. H., *J. Soc. Dyers Colourists*, **65**, 597(1949)
- (4) Lead, W. L., *ibid.*, **65**, 723(1949)
- (5) Dey, C. M., and Vaidya, B. K., *J. Indian Chem. Soc.*, **31**, 717(1955)
- (6) Moran, J. J., and Stonehill, H. I., *J. Chem. Soc.*, 765(1957)
- (7) *Ibid.*, 770(1957)
- (8) *Ibid.*, 788(1957)
- (9) Pruthi, J. S., and Lal, G., *Indian J. Hort.*, **7**, No. 2, 15(1950)
- (10) Venkataraman, K., "The Chemistry of Synthetic Dyes," Vol. 11, 1952, Academic Press, Inc., N. Y.
- (11) Geake, A., *Trans. Faraday Soc.*, **37**, 68(1941)
- (12) Appleton, D., and Geake, A., *ibid.*, **37**, 45(1941)
- (13) Krause, A., and Stasinski, H., *J. Rozzniki Chem.*, **28**, 359(1951), through *C. A.*, **49**, 7916f
- (14) Dimitrie, A. K., *Zhur. Obshchei Khim.*, **22**, 545(1952), through *C. A.*, **46**, 8477a
- (15) Kauffman, H. J., *J. Am. Chem. Soc.*, **69**, 899(1947)
- (16) Heft, H. R., and Kolb, W., Swiss Pat., 261,359, Jan. 3, 1950, through *C. A.*, **44**, 5130f
- (17) Hayashi, S., Sugino, K., and Mizuguchi, J., Japanese Pat. 176, 226, May 11, 1915, through *C. A.*, **45**, 5018b

A Bacteriological Evaluation of Boric Acid-Water-Sorbitol Solutions*

By JOHN J. SCIARRA,† DONALD E. SHAY,‡ and JOHN AUTIAN§

A study of the effect of various concentrations of sorbitol on the antibacterial activity of boric acid in aqueous solution was made. Saturated solutions of boric acid in various concentrations of sorbitol were prepared at 25°. Zones of inhibition were determined using the agar plate method and *Micrococcus pyogenes* var. *aureus* as the test organism. Bacteriostatic or bactericidal activity was also determined. The pH of all solutions was measured in order to correlate antibacterial activity with pH.

A SATURATED SOLUTION of boric acid has been used as an antiseptic for many years. Since this solution represents 5 per cent boric acid, an

increase in concentration becomes impossible. It was previously reported that sorbitol in combination with boric acid will increase the solubility of this acid from 5 per cent to approximately 20 per cent by weight (1). It was hoped that this increased concentration of boric acid would prove to be a better antiseptic solution.

The antiseptic activity of boric acid in sorbitol solutions is possibly due to three factors: the increased concentration of boric acid, the antiseptic properties of the complex compound, and the decrease in pH of the solution.

Zipf and Stephan (2) reported that products of boric acid with aliphatic polyhydroxy compounds have a lower pH than boric acid and possess higher toxic effects on organisms.

Many methods have been used to test the antibacterial power of preparations designed for application to the body surfaces (3, 4). One of these, the phenol coefficient technique, is limited

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to those substances possessing germicidal properties and is not an effective means of evaluating bacteriostatic substances (4). Such methods as: the wet filter-paper, dry filter-paper, agar-plate, serum agar-plate, agar cup-plate, etc., have been used in the past for this purpose. The agar-plate method has been used in this investigation to evaluate the boric acid-sorbitol solutions. When using the agar-plate technique, the cooled melted agar is inoculated with the test organism, in this case *Micrococcus pyogenes* var. *aureus*, poured in a Petri dish and allowed to harden. The test substance is placed into penicillinders which have been set on the surface of the agar, and the plates are then incubated at 37.5° for from twenty-four to forty-eight hours. Upon examination a clear zone around the test substance indicates no visible growth of the organism; the wider the zone the greater are the diffusion and antibacterial powers of the substance. If the substance is not inhibitory the organisms will grow over the entire plate, even in contact with the test substance. Samples of agar within the zone are then subcultured in a nutrient broth, incubated at 37.5° for forty-eight hours, and examined for turbidity. A clear broth is indicative of no bacterial growth, hence bactericidal activity; while a turbid broth indicates bacterial growth or bacteriostatic activity.

This paper presents the results of comparing the zones of inhibition manifested by boric acid alone and in combination with sorbitol.

EXPERIMENTAL

The media used in this experiment were sterile nutrient agar, pH 6.8 (B. B. L.); and sterile nutrient broth, pH 6.9 (B. B. L.).

The organism used was *Micrococcus pyogenes* var. *aureus* (F. D. A. strain 209). It was selected as the test organism since this is one of the bacteria used by the Federal Food and Drug Administration to test antibacterial substances. A 4-mm. loopful of organisms from a stock culture was transferred to a sterile tube of nutrient broth and incubated at 37.5° for twenty-four hours.

Solutions of sorbitol in distilled water were prepared containing from 0 to 70% by weight of sorbitol in increments of 5%. These solutions constituted the controls.

Solutions of boric acid in distilled water were prepared containing from 0 to 5% by weight of boric acid in increments of 0.5%.

The boric acid-sorbitol solutions were prepared according to the concentrations given in Table I.

The Petri dishes were prepared by liquefying the sterile medium, adding 1 ml. of a twenty-four-hour broth culture of the test organism when the medium cooled to 56° and then pouring into the Petri dishes in 20-ml. amounts. When the plates solidified four penicillinders were placed equidistant on the surface of the agar.

TABLE I.—CONCENTRATION OF BORIC ACID-SORBITOL SOLUTIONS

Solution No.	Boric Acid, % (w/w)	Sorbitol, % (w/w)	Water, % (w/w)
1	5.57	0 00	94 43
2	6.31	4 68	89 01
3	7.09	9 29	83 62
4	7.87	13 82	78 31
5	8.72	18 26	73 02
6	9.47	22 63	67 90
7	10.42	26 87	62 71
8	11.18	31 09	57 73
9	11.63	35 47	52 90
10	12.92	39 19	47.89
11	13.80	43.10	43 10
12	14.98	46.76	38 26
13	16.17	50.30	33 53
14	17.31	53 75	28 94
15	18 66	56 96	24 38

By means of sterile pipets five drops of each test solution were placed into the penicillinders on the surface of the Petri dishes. The dishes were then incubated at 37.5° for forty-eight hours. The size of the zone was recorded as the distance between the outer edge of the penicillinders and the periphery of the clear zone.

A sample of the agar in the clear zone was then subcultured in nutrient broth and incubated at 37.5° for forty-eight hours.

RESULTS

The zone of inhibition manifested by boric acid in sorbitol solutions is shown in Table II. These results are shown graphically in Fig. 1. Table III and Fig. 2 show the results obtained using solutions of boric acid in distilled water.

TABLE II.—ANTIBACTERIAL EFFECT OF BORIC ACID IN SORBITOL SOLUTION

Solution No.	Zone of Inhibition, mm				pH
	Detn No 1 ^a	Detn No 2 ^a	Detn No 3 ^a	Av	
1	4.3	8 1	8 8	7 1	3 80
2	4.4	9 0	8 4	7 3	2 90
3	6 9	7 6	8 3	7 6	2 50
4	5.4	8 3	5 4	6 4	2 35
5	5.6	8 0	4 9	6 2	2 20
6	6.5	8 0	5.9	6 8	2.15
7	6.6	8 1	5 3	6 7	2.10
8	5.6	7 4	5 1	6 0	2 00
9	7.1	7 9	6 1	7.1	1.90
10	8 2	7.4	7 1	7.6	1 85
11	6.3	8 3	6 9	7.2	1 75
12	6.3	8.3	7 1	7 2	1.70
13	6.0	8 9	7 4	7 4	1 70
14	6.0	9.4	7.3	7 3	1 60
15	7.1	9.5	8.1	8.1	1.60

^a Average of eight determinations.

The control solutions of sorbitol alone did not inhibit the growth of *Micrococcus pyogenes* var. *aureus*. This observation is not surprising since

TABLE III.—ANTIBACTERIAL EFFECT OF BORIC ACID IN WATER

Boric Acid, Concn., % w/w	Average Zone of Inhibition, ^a mm	pH
0.0	0.0	7.20
0.5	2.0	6.35
1.0	3.4	5.85
1.5	4.6	5.50
2.0	5.9	5.10
2.5	6.4	4.85
3.0	6.8	4.55
3.5	7.9	4.45
4.0	8.6	4.30
4.5	8.9	4.15
5.0	9.7	3.95

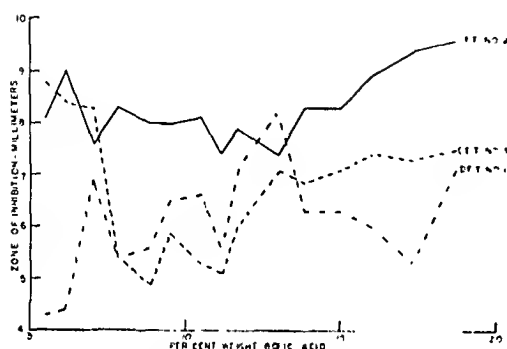
^a Average of eight determinations.

Fig. 1—Antibacterial activity of boric acid-sorbitol solution.

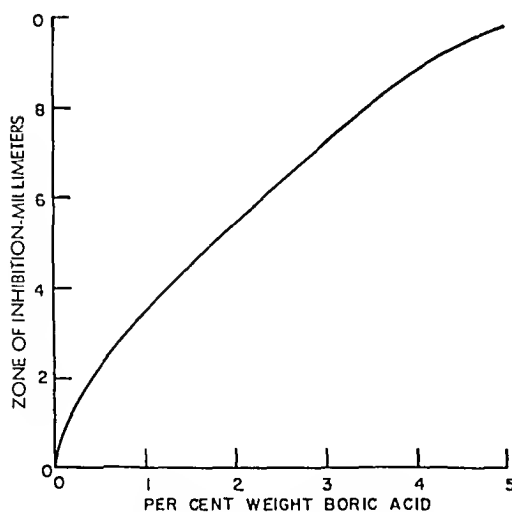


Fig. 2—Antibacterial activity of boric acid in water.

sorbitol is not antibacterial, in fact, it is sometimes used as a component of various media used for the growth of bacteria.

The results of the subculture of the clear zone of inhibition in nutrient broth showed boric acid in these concentrations, and when tested in this manner, exerted a bacteriostatic effect and not a bacteri-

cidal one. All of the tubes indicated bacterial growth after a forty-eight hour incubation period.

DISCUSSION OF RESULTS

The results suggest that the maximum antibacterial activity of boric acid occurs when used in a concentration of approximately 5%. Any further increase in concentration of boric acid, made possible by the addition of sorbitol, was not indicative of greater antibacterial activity. Apparently the sorbitol interferes in some manner with the antimicrobial activity of boric acid. No direct relationship was found to exist between the concentration of boric acid in sorbitol solution and the antibacterial effect. There were several solutions where the effectiveness decreased with an increase in concentration of boric acid. This decrease may be due to the formation of a complex compound between boric acid and sorbitol. The antibacterial activity of this new compound has been found to be less than that of a 5% boric acid solution in water.

In an attempt to understand the cause for the variation in activity of boric acid in the presence of sorbitol, the pH of the solution was measured. The pH was found to decrease with an increase in concentration of boric acid. This lowering of pH verified the presence of a more acidic compound but did not explain the various peaks shown in Fig. 1. Had there been an increase in pH for those solutions showing lesser antibacterial activity, the difference could be explained. It is known that a decrease in hydrogen-ion concentration for acidic substances will decrease antibacterial activity and an increase in hydrogen ion concentration will increase antibacterial activity (5).

A possible explanation, at this time, for these differences in antibacterial activity of boric acid solutions in the presence of sorbitol, may be that sorbitol, being a nutrient for bacteria, hinders the activity of boric acid. Further studies are indicated to explain the mechanism of this activity.

SUMMARY AND CONCLUSIONS

1. A study was made of the antibacterial activity of various solutions of boric acid in the presence of sorbitol.
2. Maximum antibacterial activity of boric acid occurred at about a 5 per cent concentration.
3. No relationship was found to exist between the concentration of boric acid in sorbitol solution and the antibacterial activity, nor between the pH and antibacterial activity. Further work is necessary to fully establish these observations.
4. Boric acid in sorbitol solution was found to be bacteriostatic rather than bactericidal.

REFERENCES

- (1) Scierra, J. J., Antian, J., and Foss, N. E., *This Journal*, 47, 144 (1958). A. Ph. A. New York meeting, April 1957.
- (2) Zipf, H. F., and Stephan, G., *Arch. exp. Pathol. Pharmacol.*, 207, 55 (1949); *Chem. Abstr.*, 46, 9710 (1952).
- (3) Kelly, F. C., and Hile, K. E., "Microbiology," Appleton-Century-Crofts, Inc., New York, 1949, p. 228.
- (4) Ruelke, G. L., and Brower, C. M., *Bull. No. 98 U. S. Food and Drug Administration*.
- (5) Kelly, F. C., and Hile, K. E., "Microbiology," Appleton-Century-Crofts, Inc., New York, 1949, p. 218.

The Toxicity and Metabolism of Dihydroquercetin*

By ALBERT N. BOOTH and FLOYD DeEDS

Dihydroquercetin, which is closely related to quercetin in chemical structure, has been shown to be nontoxic when fed to albino rats at a dietary level of 1 per cent for long periods of time. Chromatographic examination of the urine of two human volunteers before and after the ingestion of 2 grams of dihydroquercetin demonstrated the conversion of the compound to 3,4-dihydroxyphenylacetic, *m*-hydroxyphenylacetic, and 3-methoxy-4-hydroxyphenylacetic acids. These same metabolites are excreted following oral administration of quercetin, or DOPA, to rats, rabbits, or humans.

THE ANTIOXIDANT PROPERTIES, physiological effects, and metabolic fate of rutin and its aglycone, quercetin, have been studied in this laboratory (1-8). The close structural relation of dihydroquercetin to quercetin (Fig. 1) invites comparison of the two compounds in regard to toxicity, antioxidant properties, physiological effects, and metabolism.

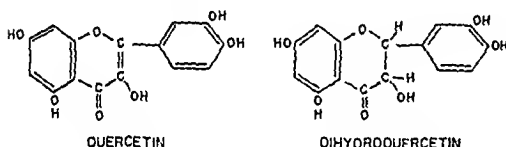


Fig. 1.—Structural formulas of quercetin and dihydroquercetin.

Quercetin occurs as the glycoside rutin in the buckwheat plant (*Fagopyrum esculentum* and *tartaricum*), in the leaves of *Eucalyptus macrorhyncha*, in the flower buds of the Chinese scholar tree (*Sophora japonica*), and as the rhamnoside quercitrin in the bark of the black oak tree (*Quercus velutina*). Commercial quercetin has been prepared by acid hydrolysis of the material isolated from these sources (9-12). At present the most prolific source of dihydroquercetin is Douglas fir bark which contains 80 to 152 pounds per ton of bark (13). The ease of conversion of dihydroquercetin to quercetin (14) makes Douglas fir bark another potentially important source of the latter.

In a study of comparative abilities of a number of flavonoids to protect epinephrine against oxidation *in vitro* Wilson and DeEds (3) reported that 3 molar equivalents of dihydroquercetin pro-

vided about 0.8 the antioxidant effect afforded by 1 molar equivalent of quercetin. Because of the ease with which dihydroquercetin is oxidized to quercetin it was recently suspected that the antioxidant activity *in vitro* ascribed to dihydroquercetin may have been due to quercetin present as an impurity. Wilson has now shown that dihydroquercetin purified by repeated crystallization has negligible antioxidant protective action toward epinephrine *in vitro* (15). This does not necessarily militate against the possibility that dihydroquercetin may be converted to quercetin in the animal body and therefore produce the same physiological effects as quercetin. The observations on the metabolism of dihydroquercetin reported herein are in harmony with this suggestion. Moreover, Ambrose, Robbins, and DeEds (6) reported that dihydroquercetin, in common with rutin, quercitrin, and quercetin, provided some protection against severe acute frostbite in rabbits. Unpublished data obtained on 5 rabbits, using the technic described by Ambrose and DeEds (2, 4) demonstrated that dihydroquercetin decreased the responsiveness of cutaneous capillaries to acute local irritation.

EXPERIMENTAL

Chronic Toxicity.—For comparison with toxicity data reported on quercetin (7), the following long-term feeding experiment with dihydroquercetin was conducted. Twenty weanling albino rats of each sex from our stock colony served as controls on a basal diet having the following percentage composition: degerminated yellow corn meal, 73; crude casein, 10; linseed oil cake meal, 10; ground alfalfa, 2; bone ash, 1.5; sodium chloride, 0.5; and cod liver oil, U. S. P. 3. Ten weanling rats of each sex of the same strain were placed on dietary levels of 0.125, 0.25, 0.5, and 1.0% of dihydroquercetin incorporated in the same basal diet. Groups of five rats, all of one sex, were kept in cages with pine shavings as litter, and with free access to water and food at all times. The amount of dihydroquercetin available dictated the following feeding periods. At the end of 226 days, half of the animals receiving 1.0 and 0.5% of the compound and an equal number of controls were sacrificed; the remainder on these concentrations being sacrificed after 450 days. After 249 days, half of the animals on diets containing 0.25 and 0.125% and an equal number of controls were sacrificed, the remainder being continued for 650 days. The range of dosage levels employed was deemed adequate in view of the close chemical relationship of dihydroquercetin to quercetin and

* Received May 28, 1957, from Pharmacology Section, Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, Calif.

the reported lack of toxicity of the latter (7) at a dietary level of 1%.

At weekly intervals throughout the course of the experiment the rats were weighed individually and carefully examined. At this time the food consumption of each group of five rats was determined.

Throughout the course of the feeding test no abnormalities in appearance or behavior of the experimental animals as compared with the controls were observed. A rather high incidence of respiratory infection, as evidenced by rales, developed in the control animals as well as in those on the various dietary levels of dihydroquercetin. There were 12 deaths in a total of 120 rats during the experiment, but the deaths bore no relation to the treatment. As compared with the controls, the various dosage levels of dihydroquercetin had no effect on either food intake or rate of growth. The lack of any effect on growth is shown in Table I, which summarizes the average initial and final weights of rats on the control and experimental diets at the end of 226 days. No differences were found in body length or weights of liver, kidney, spleen, heart, adrenal, and testis between animals receiving 1% dihydroquercetin for 226 days and their appropriate controls.

TABLE I—BODY WEIGHT GAIN OF RATS ON CONTROL AND DIHYDROQUERCETIN-CONTAINING DIETS FOR 226 DAYS

%	Males		Females	
	Initial Av. Wt., Gm.	Final Av. Wt., Gm.	Initial Av. Wt., Gm.	Final Av. Wt., Gm.
Control	40	362	42	251
0.125	39	352	37	251
0.25	39	359	37	258
0.5	42	356	46	246
1.0	42	361	46	248

At 450 days five control rats of each sex, five males and four females receiving 1%, and four males and five females receiving 0.5% dihydroquercetin were sacrificed and examined for gross abnormalities. Paraffin sections of kidney, liver, heart, lung, testis, ovary, thyroid, hypophysis, adrenal, gut, pancreas, spleen, and bladder were stained with hematoxylin-eosin for histopathological examination. Lung consolidation was observed grossly in one control rat of each sex, in one rat of each sex receiving 1%, and in one female receiving 0.5% dihydroquercetin. Cysts were found on the liver and kidney of one control female, and on the uterus and ovary of another control. Bladder stones were found in the male controls and a kidney tumor in one male control.

Histopathological examination of the stained tissue sections showed that a majority of the control rats had mild focal pyelonephritis. The kidney tumor noted grossly in a male control was a tubular adenoma of questionable malignancy. The lung consolidations noted grossly were suppurative, and the cysts were of inflammatory origin. No changes of significance were found in any of the organs of male rats fed 0.5 and 1.0% dihydroquercetin, nor were any significant changes found in other organs of female rats fed 0.5% dihydroquercetin. The

livers of the female rats fed 1.0% of the compound showed vacuoles, probably due to fat deposition, scattered throughout. With the possible exception of this vacuolization, neither the gross nor microscopic examination revealed any significant changes attributable to the dihydroquercetin.

Metabolic Fate.—Booth, Murray, Jones, and DeEds (8) have demonstrated that oral administration of quercetin to rats, rabbits, quinea pigs, and humans results in the urinary excretion of 3,4-dihydroxyphenylacetic acid, *m*-hydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid). It has also been shown by DeEds, Booth, and Jones (16) that these same metabolites are excreted following oral administration of DOPA (3,4-dihydroxyphenylalanine).

When the urine of two human volunteers was examined by the chromatographic procedures described previously (8), before and after the ingestion of 2 Gm. of dihydroquercetin, it was found that the same metabolites were excreted as in similar experiments with quercetin and with DOPA.

SUMMARY

No significant toxic results were observed from dihydroquercetin,¹ which is closely related to quercetin in chemical structure, when fed to albino rats at a dietary level of 1% for long periods of time. It has also been shown that the metabolic fate of dihydroquercetin is the same as that of quercetin and DOPA. It is tempting to speculate that the lack of toxicity of both quercetin and dihydroquercetin is due to the fact that both compounds serve as substrates in the same metabolic pathways that account for the metabolism of DOPA.

REFERENCES

- (1) Wilson, R. H., Mortarotti, T. G., and DeEds, F., *J. Pharmacol. Exptl. Therap.*, **90**, 120 (1947).
- (2) Ambrose, A. M., and DeEds, F., *ibid.*, **90**, 359 (1947).
- (3) Wilson, R. H., and DeEds, F., *ibid.*, **95**, 399 (1949).
- (4) Ambrose, A. M., and DeEds, F., *ibid.*, **97**, 115 (1949).
- (5) Ambrose, A. M., and DeEds, F., *J. Nutrition*, **38**, 305 (1949).
- (6) Ambrose, A. M., Robbins, D. J., and DeEds, F., *Federation Proc.*, **9**, No. 1 (1950).
- (7) Ambrose, A. M., Robbins, D. J., and DeEds, F., *This Journal*, **41**, 110 (1952).
- (8) Booth, A. N., Murray, C. W., Jones, F. T., and DeEds, F., *J. Biol. Chem.*, **223**, 251 (1956).
- (9) Coneh, J. F., Krewson, C. F., and Naghski, J., U. S. *Dept. of Agriculture and Ind. Chem. Research Laboratory* July 1947.
- (10) Coneh, J. F., Krewson, C. F., and Naghski, J., *ibid.*, AIC-202 (Eastern Regional Research Laboratory) July 1948 (Processed).
- (11) Coneh, J. F., Naghski, J., and Krewson, C. F., *J. Am. Chem. Soc.*, **74**, 424 (1952).
- (12) Booth, A. N., and DeEds, F., *This Journal*, **40**, 384 (1951).
- (13) Kurth, E. F., and Chan, F. L., *J. Am. Oil Chem. Soc.*, **28**, 433 (1951).
- (14) Kurth, E. F., *J. Ind. Eng. Chem.*, **45**, 2096 (1953).
- (15) Unpublished data.
- (16) DeEds, F., Booth, A. N., and Jones, F. T., *J. Biol. Chem.*, **225**, 615 (1957).

¹ It is a pleasure to thank the Oregon Forest Products Laboratory for a liberal supply of dihydroquercetin which made this investigation possible.

Catalase in *Cunninghamella Blakesleeana* H-334*

By ARLINGTON A. FORIST

Techniques for the determination of crystalline catalase activity have been extended to crude cell homogenates and employed in an examination of catalase in *Cunninghamella blakesleeana* H-334. Variation in catalase content during growth of this microorganism indicates that the older, mature cells are the catalase producers. Catalase does not appear to be involved in the 11 β -hydroxylation of steroids by *Cunninghamella blakesleeana* H-334.

CUNNINGHAMELLA BLAKESLEEANA strain H-334 (ATCC 9245) contains enzyme systems capable of directly introducing the 11 β -hydroxyl group, necessary for biological activity, into the steroid nucleus. Incubation of Reichstein's compound S (11-desoxy-17 α -hydroxycorticosterone) with this microorganism yields hydrocortisone (17 α -hydroxy-corticosterone) (1). An examination of enzymes present in this microorganism has included a study of its catalase. The present report describes the method of determination and some of the properties of catalase in *Cunninghamella blakesleeana* H-334 along with the change in catalase content during growth of the microorganism.

Bonnichsen, Chance, and Theorell (2) have described an excellent method for the measurement of crystalline catalase activity based on a high enzyme-low substrate ratio and a short reaction time. By this procedure, disappearance of hydrogen peroxide was observed to follow a first order rate. The present communication describes an extension of this technique to the measurement of catalatic activity in crude systems.

EXPERIMENTAL

Cultures.—Most of the *C. blakesleeana* H-334 samples examined were grown in shake flasks on a complex medium (soybean meal, 5 Gm.; brewer's yeast, Pabst, 5 Gm.; dextrose, 20 Gm.; NaCl, 5 Gm.; KH₂PO₄, 5 Gm.; tap water, 1,000 cc.; pH adjusted to 6.4) as described by Mann, *et al.* (3). Some samples were also grown on Czapek-Dox solution (NaNO₃, 3.0 Gm.; K₂HPO₄·3H₂O, 1.3 Gm.; MgSO₄, 0.5 Gm.; KCl, 0.5 Gm.; FeSO₄·7H₂O, 0.01 Gm.; sucrose, 30 Gm.; distilled water, 1,000 cc.; pH adjusted to 7.2) (4).

Preparation of Crude Catalase.—The mycelia of *C. blakesleeana* H-334, usually pellet-like, were

separated from the medium by filtration on cheesecloth or by centrifugation, washed with distilled water on the filter or by centrifugation, and resuspended in cold *M*/15 phosphate buffer, pH 7.0. The cell suspension was then homogenized with a pestle homogenizer or for five minutes in a Waring Blendor. The resulting homogenate was the crude enzyme.

Measurement of Catalatic Activity.—An aliquot of the crude enzyme solution, usually 5 cc., was mixed with sufficient *M*/15 phosphate buffer, pH 7.0, to make a total of 48 cc. of solution in a 29 x 200 mm. test tube. The resulting solution was cooled to 0° in a crushed ice-water bath (Dewar flask). A 2-cc. portion of 0.25 *N* hydrogen peroxide, also at 0°, was blown from a volumetric pipet into the reaction mixture, the stop watch started, and the solution thoroughly mixed. Five 5-cc. aliquots of the reaction mixture were withdrawn in rapid succession over a period of two to three minutes and added to 5-cc. portions of 2 *N* sulfuric acid. The time at which the meniscus passed an etched line on the pipet bulb was recorded for each sample. The hydrogen peroxide remaining at each time was determined by adding 10 cc. of 10% potassium iodide and a drop of 1% ammonium molybdate to the acidified samples, and, after three minutes, titrating the liberated iodine to a starch endpoint with 0.005 *N* sodium thiosulfate.

Attempts to titrate residual hydrogen peroxide with potassium permanganate were unsuccessful due to consumption of the oxidant by cellular debris. However, the iodometric procedure (5) gave satisfactory results. The allowable variation in the time of titration of liberated iodine following addition of potassium iodide to the acidified samples was tested. Reduction of hydrogen peroxide was complete after two minutes, but a gradual evolution of iodine, due to air oxidation of iodide ion, continued. Therefore, titrations were routinely made between three and five minutes after addition of the potassium iodide.

Calculation of Results.—No deviations from a first order disappearance of hydrogen peroxide were observed. In every case a plot of the common logarithm of the sodium thiosulfate titer *vs.* time of quenching of the enzymatic reaction in acid gave a straight line. The exact normalities of the reagents involved were not required. Typical rate curves are shown in Fig. 1. The first order pseudo constant, *k*, the slope of the rate curve, was directly proportional to the catalase content as indicated in Table I. Therefore, results have been expressed in terms of *k* evaluated under the conditions described above. Actually, *k* is the product of the specific reaction rate constant and the catalase concentration.

Variation in Catalase Content with Growth of the Microorganism.—A typical experiment in which variation in catalase content with growth of *C. blakesleeana* H-334 was evaluated is presented. A total of 400 cc. of seed grown sixty-six hours in

* Received May 31, 1957, from the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

The author is indebted to E. J. Collins for the nitrogen determinations.

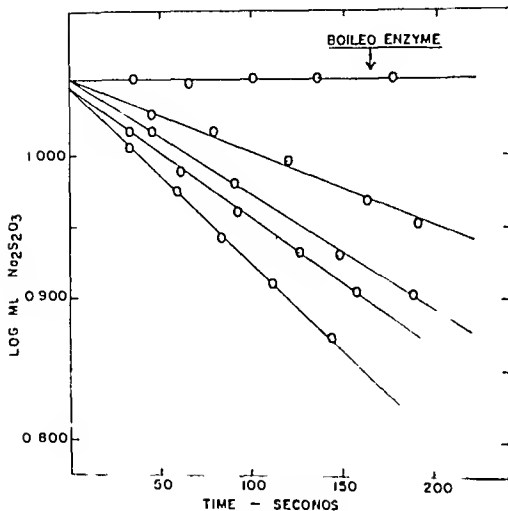


Fig 1 Typical rate curves

TAB. I —RELATIONSHIP OF PSEUDO CONSTANT TO CATALASE CONCENTRATION

Homogenate (cc)	$k \times 10^3(\text{sec}^{-1})$	$k/\text{cc} \times 10^3(\text{sec}^{-1})$
2	51.0	25.5
3	80.3	26.8
1	91.0	22.8
5	123.0	24.6
Av. 21.9 ± 0.9		

shake flasks on Czapek Dox solution with 0.3% added aspartic acid was added to a 12 x 12 inch battery jar fermenter containing 8 L. of Czapek Dox solution with 0.1% added aspartic acid. The mixture was agitated at 275 r.p.m. and aerated at 0.5 L. of air per minute at 29-32°. Periodically, aliquots of the cell suspension were removed and the catalase content determined as outlined above. Nitrogen content of the cells was determined by a micro Kjeldahl procedure.

RESULTS AND DISCUSSION

Preparation of Crude Enzyme.—Results of a series of determinations designed to indicate the best method of preparation of the crude catalase are shown in Table II. From these data it can be concluded that (a) little activity is measurable without some type of cell rupture, (b) homogenization in distilled water or buffer is equally effective, (c) buffer tends to extract more of the enzyme into solution, and (d) pestle homogenization following treatment in a Waring Blendor does not liberate additional enzyme. In routine determinations, pestle homogenization was selected because the resulting solutions were easier to pipet, and buffer was employed since Svendsen (6) has suggested that the presence of salts lowers catalase loss due to adsorption on glass surfaces.

Properties of the Crude Catalase.—Activity of the crude catalase from *C. blakesleeana* H-334 is independent of pH over the range 6.2-7.7. The crude enzyme is stable for at least two days when stored under refrigeration. Catalatic activity is

TAB. II —VARIATION IN ACTIVITY OF CRUDE CATALASE WITH METHOD OF PREPARATION

Expt	Preparation	$k \times 10^3(\text{sec}^{-1})$
1a	Whole cells	8
1b	Homogenate of 1a (Waring Blendor 3 min.)	59
2a	Cell homogenate (Waring Blendor 5 min., distilled water)	67
2b	Cell homogenate (Waring Blendor 5 min., phosphate buffer)	70
2c	Supernatant from 2a	2
2d	Supernatant from 2b	28
2e	Precipitate from 2b	42
2f	2c resuspended and pestle homogenized	45

destroyed by boiling (Fig. 1) and is inhibited by 2,4-dichlorophenol (7). Electron bombardment of whole cells (2,000,000 r.e.p.-35 seconds) does not reduce the catalase content.

Variation in Catalase Concentration with the Growth of *C. Blakesleeana* H-334.—The change in catalase concentration during the growth of *C. blakesleeana* H-334 on a modified Czapek Dox medium is shown in Fig. 2 along with the change in

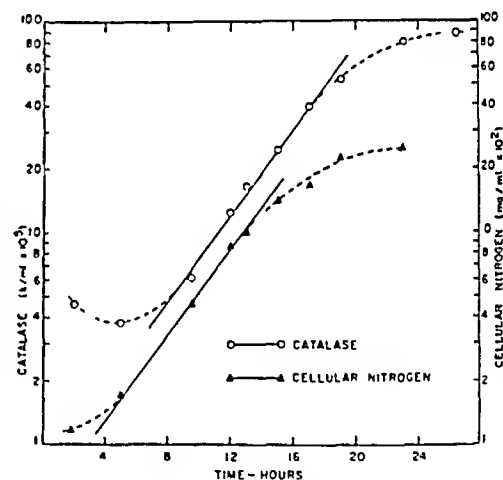


Fig 2—Variation in catalase content and cellular nitrogen during growth of *C. blakesleeana* H-334 on a synthetic medium (Czapek-Dox solution plus 0.1% aspartic acid)

cellular nitrogen. Catalase concentration follows a typical S-shaped growth curve. During the logarithmic phase, both catalase and cellular nitrogen increase at approximately the same rate. However, this logarithmic phase begins considerably later in the case of catalase and continues for some time after cellular nitrogen has begun to plateau. Apparently the older, mature cells are the catalase producers. The higher catalase concentration early in the growth period is believed due to the presence of the mature, catalase-rich cells from the seed. Nielson (8) has reported that young cells of *C. blakesleeana* H-334 are the most active in the conversion of 11-desoxy-17 α -hydroxycorticosterone to

17 α -hydroxycorticosterone. It therefore appears that catalase is not involved in this transformation.

SUMMARY

The technique of Bonnicksen, Chance, and Theorell (2) has been extended to catalase measurements in crude cell homogenates. Properties of the catalase of *Cunninghamella blakesleeana*, strain H-334, have been examined. Variation in catalase content of cells during the growth of the microorganism has been determined.

REFERENCES

- (1) Hanson, F. R., Mann, K. M., Nielson, E. D., Anderson, H. V., Brunner, M. P., Karnemaat, J. N., Colingsworth, D. R., and Haines, W. J., *J. Am. Chem. Soc.*, **75**, 5369 (1953).
- (2) Bonnicksen, R. K., Chance, B., and Theorell, H., *Acta Chem. Scand.*, **1**, 685 (1947).
- (3) Mann, K. M., Hanson, F. R., O'Connell, P. W., Anderson, H. V., Brunner, M. P., and Karnemaat, J. N., *Appl. Microbiol.*, **3**, 14 (1955).
- (4) O'Connell, P. W., Mann, K. M., Nielson, E. D., and Hanson, F. R., *ibid.*, **3**, 16 (1955).
- (5) Sumner, J. B., and Somers, G. F., "Chemistry and Methods of Enzymes," Academic Press Inc., New York, 1947, p. 209.
- (6) Svendsen, A., *Acta Chem. Scand.*, **7**, 551 (1953).
- (7) Goldacre, P. L., and Galston, A. W., *Arch. Biochem. Biophys.*, **43**, 169 (1953).
- (8) Nielson, E. D., Division of Agricultural and Food Chemistry, 126th Meeting, A. C. S., New York, 1954.

Investigation of Human Skin Lipids I*

By JAMES E. TINGSTAD, DALE E. WURSTER, and TAKERU HIGUCHI

A method of harvesting relatively large quantities of skin lipids from human volunteers is presented. Experimental evidence indicate that the collected lipids are composed mainly of those produced by the sebaceous glands and that the lipids obtained from the arm and back regions of the body are similar in chemical composition. No significant chemical decomposition of the skin lipids was detected when the material was stored at low temperatures. The chemical species which are responsible for observed seasonal variations in the composition of human skin lipids have been determined.

THE MECHANISM whereby drugs penetrate the skin following external application has been under investigation for several decades. It is generally accepted that three possible routes of penetration exist: (1) along ducts of eccrine sweat glands into the glands and then into the surrounding tissue fluid, (2) directly through the layers of skin, and (3) down hair follicles and sebaceous ducts, then through the sebaceous glands and into the surrounding tissue fluid.

Both Rothman (1) and MacKee (2) have indicated the importance of the follicular pathway in the penetration of skin by drugs. In later work, MacKee, *et al.* (3), working with dyes, sulfas, and heavy metals, indicated that most of the penetration by these agents occurred via the follicular route. More recently it has been stated (4) that perifollicular whealing follows the application of histamine to the skin. The latter strongly suggests that transfollicular penetration occurs.

If the follicular route is an important one in the absorption of drugs through the skin, it is likely that skin lipids, both in the lipid surface film and in the follicles, play an important part in determining speed and extent of absorption. It would thus be desirable, as an initial step in a broad study of skin penetration, to determine the behavior of drugs in skin lipids. In order to make these extensive *in vitro* studies, adequate amounts of human skin lipids must first be collected.

This paper presents (a) a method of harvesting skin lipids from human volunteers on a large scale; (b) a study of the variations in the chemical composition of the collected material as a function of the method employed, the time of the year, and the body area extracted; and (c) the results of an investigation of the chemical nature of the components responsible for the observed variation.

EXPERIMENTAL

Collection of Skin Lipids.—The skin lipids were obtained from human volunteers by means of direct extraction from the arms with a volatile solvent.

Mass lipid extractions were made at irregular intervals over a period of nearly two years, during which time the skin lipids from the arms of more than 1,000 student volunteers were obtained. Each

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Based on a dissertation submitted by James E. Tingstad to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

mass extraction contained the pooled lipids of approximately 50 subjects

Solvent.—Ethyl ether (reagent grade), which was distilled just before use to avoid the dangers associated with peroxide formation, was used as the extracting medium

Collection Procedure.—The hands and arms of each subject were washed with a sodium lauryl sulfate solution, rinsed with distilled water, and dried. The hands were then immersed in ether for thirty seconds to insure complete removal of contaminants from this area. This portion of the ether wash was discarded.

The hand and arm were then immersed in the purified ether (1,750 ml.) contained in a glass tube 100 mm. in diameter and 56 cm. high. A wooden block was used to hold the extraction tube in place. The arm was maintained in the tube for one minute; the procedure was then repeated on the other arm. The ether in the tube was replaced after 15 subjects had been subjected to the extraction procedure.

The lipids were recovered from the ether solution by distilling off the solvent. When the volume was sufficiently reduced (150 ml.), the concentrated lipid solution was filtered through a medium porosity, sintered glass filter. The clear filtrate was then carefully heated, with agitation, under a stream of nitrogen to remove all traces of solvent.

Analysis of the Lipids.—The gross chemical composition of each sample of collected skin lipids was determined by infrared analysis and by acid and iodine number determinations. Infrared spectra were obtained on a Baird Associates infrared spectrophotometer, employing a sodium chloride prism and carbon tetrachloride as the solvent. Acid numbers were determined by the U. S. P. XV method, except that 0.01 N potassium hydroxide was used as the standard base. Iodine numbers were determined by the method of Yasuda (5), except that 0.2 N pyridine sulfate dibromide solution was used as the halogenating agent.

Effect of Extent of Extraction on Composition.—It was desirable to determine whether the extracted lipids were essentially those being produced by the sebaceous gland. For this reason lipids from the arms of four subjects were extracted in the usual manner. After a two-hour interval, during which time great care was taken to avoid contamination of the hands or arms, the arms were again immersed in a new supply of ether for two and one-half minutes. The extracts were then analyzed chemically and by infrared.

For another series of experiments, the arms of an individual were extracted by the standard procedure at two-day intervals for a period of six days. The material collected was then subjected to infrared analysis and acid number determination.

Lipids from the Arm and the Back.—In order to determine whether the lipid samples obtained from the arm were similar to the skin lipids from other parts of the body, both spectrophotometric and chemical comparison of the lipids from the arm were made with lipid samples from the back. Since extraction of lipids from the back by immersion was not possible, a modified procedure was followed.

The back of each subject was washed and dried in the usual manner. The subjects were then placed in a prone position, and glass cylinders 3.8 cm. in diameter, 10 cm. high, and open at both ends were then

held firmly against the skin of the back. About 75 ml. of ether was then placed in the cylinder and the cylinder was carefully moved over the skin surface. The time of exposure to the solvent for each area was approximately fifteen seconds. After the extraction period, the lipid solution was removed from the cylinder with a pipet. The lipids were then recovered in the previously described manner. Extraction from the arms of the subject from the elbows to the shoulders was made by the same procedure. The gross composition of the two lipid samples was determined in the usual manner.

Deterioration During Storage.—To determine the effects of storage on skin lipids, two portions of a sample of lipids were stored under different conditions: one in a refrigerator (5–10°), and the other in a Dewar flask filled with dry ice (–80°). Chemical and spectral analyses were performed at the end of six and fifteen weeks.

Chemical Composition of Skin Lipids.—Although considerable research effort has already been directed toward the determination of skin lipid composition by others (6), it was necessary to establish the chemical nature of the component or components responsible for the observed variations in lipid composition. A liquid-liquid countercurrent extraction technique (7, 8) was therefore employed to fractionate the skin lipids.

Approximately one gram of the lipids was dissolved in petroleum ether (b. p. 35–38°) and subjected to 100 transfer stages in an all-glass, Craig-type extractor (10/10). Petroleum ether was used as the moving phase and methanol containing 2 1/2% (v/v) water as the stationary phase. After the 100th transfer, the contents of each tube were transferred to a tared aluminum moisture pan and allowed to evaporate to dryness. The solid content of each pan was then determined and infrared analyses were done on fractions of interest.

RESULTS AND DISCUSSION

Collection of Skin Lipids.—By using the described extraction procedure, it was possible to make extractions from the arms of approximately 60 to 70 people in three hours. The yield averaged 0.1 Gm. per person, a single mass extraction thus supplied about 6–7 Gm. of material. In all, over 100 Gm. of skin lipids were collected. The lipids were light brown in color and had a semisolid consistency at room temperature.

Seasonal Variation in Lipid Composition.—Infrared analysis of each of the lots of lipids indicates a possible seasonal variation in composition. Infrared spectra of typical lipid lots are shown in Figs. 1–3. The tracings are of little value in determining the absolute chemical composition of the gross mixture; however, they are useful both in establishing the relative constancy of each mixture and in detecting large variations in the amounts of different functional groups present.

Close examination of a large number of these tracings revealed that although successive samples collected over a relatively short period of time (one to two months) were quite similar, there occurred throughout the year a considerable variation in the relative amounts of the materials causing the 5.75-micron and the 5.85-micron absorption peaks. In certain lots the absorbance at 5.75 microns was greater; in others the two absorbances were about

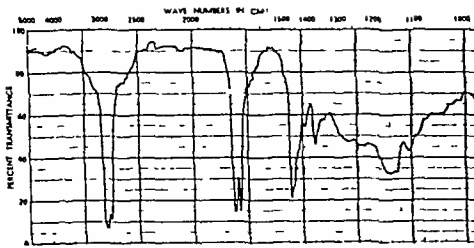


Fig. 1.—Typical infrared spectrum of lipids collected in February, March, and April, 1955.

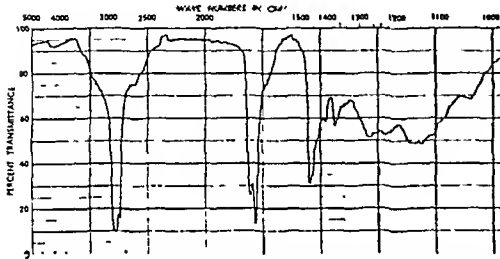


Fig. 2.—Typical infrared spectrum of lipids collected in August, September, and October, 1955.

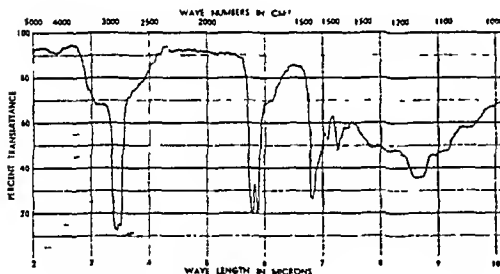


Fig. 3.—Typical infrared spectrum of lipids collected in January and February, 1956.

equal; in still others the absorbance at 5.85 microns was greater.

These variations are apparent in the data shown in Table I where the ratios of the absorbances $A_{5.75}/A_{5.85}$ for 25 different lots of skin lipids ranging from a low of 0.62 to 1.06 are given. The seasonal dependency of this ratio as indicated by the table is more apparent in Fig. 4 where it has been plotted against the time of collection.

The data suggest that the relative concentrations of the substances causing the 5.75-micron peak reach a maximum during late winter and early spring and a minimum during the months of August and September—the 5.85-micron substance following a converse pattern. This pattern of annual rise and fall is manifested during both years encompassed in the present study. This biochemical response may possibly be due to changes in the thermal environment, the diet of the volunteers during the course of the year, the presence of lipases elaborated by microorganisms, or a combination of these factors.

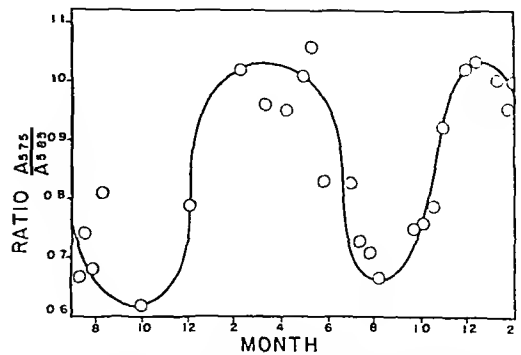


Fig. 4.—A plot showing the seasonal dependency of the absorbance ratio of the 5.75 micron to the 5.85 micron peak in the infrared (lipids from mass collections). See Table I.

Since each sample represented total lipids collected from about 50 subjects, it was believed that the effects of variations among individuals were not reflected in the data shown. This assumption was verified by work done later on individuals. Results of that study will be presented in a subsequent paper.

Acid numbers obtained for some of the lipid samples are presented in Table I. Analyses were not made on every sample, since some lots were collected specifically for other studies. In this table the acid numbers of the lipid samples subjected to analysis appear to vary with the infrared absorbance

TABLE I.—RESULTS OF ANALYSIS OF MASS EXTRACTATIONS

Date	Infrared Peak Ratio, $A_{5.75}/A_{5.85}$	Acid No.	Iodine No
7/6/54			
7/10	0.67		
7/16	0.74	73	52
7/28	0.68	93	60
8/9	0.81	78	54
10/1	0.62	109	54
12/1	0.79	61	60
2/9/55	1.02	49	68
3/9	0.96	56	60
4/6	0.95	62	69
4/28	1.01
5/11	1.06
5/25	0.83
6/29	0.83	77	50
7/13	0.73	88	42
7/27	0.71	98	43
8/10	0.67	82	52
9/21	0.75
10/5	0.76
10/19	0.79
11/2	0.92
11/30	1.02
12/14	1.03
1/11/56	1.00
1/25	0.95
2/8	1.00

ratio discussed above. This is more evident in Fig. 5 where the acid number has been plotted against the reciprocal of the ratio. The data suggest that the component or components responsible

mass extraction contained the pooled lipids of approximately 50 subjects

Solvent.—Ethyl ether (reagent grade), which was distilled just before use to avoid the dangers associated with peroxide formation, was used as the extracting medium

Collection Procedure.—The hands and arms of each subject were washed with a sodium lauryl sulfate solution, rinsed with distilled water, and dried. The hands were then immersed in ether for thirty seconds to insure complete removal of contaminants from this area. This portion of the ether wash was discarded.

The hand and arm were then immersed in the purified ether (1,750 ml) contained in a glass tube 100 mm in diameter and 56 cm high. A wooden block was used to hold the extraction tube in place. The arm was maintained in the tube for one minute, the procedure was then repeated on the other arm. The ether in the tube was replaced after 15 subjects had been subjected to the extraction procedure.

The lipids were recovered from the ether solution by distilling off the solvent. When the volume was sufficiently reduced (150 ml), the concentrated lipid solution was filtered through a medium porosity, sintered glass filter. The clear filtrate was then carefully heated, with agitation, under a stream of nitrogen to remove all traces of solvent.

Analysis of the Lipids.—The gross chemical composition of each sample of collected skin lipids was determined by infrared analysis and by acid and iodine number determinations. Infrared spectra were obtained on a Baird Associates infrared spectrophotometer, employing a sodium chloride prism and carbon tetrachloride as the solvent. Acid numbers were determined by the U. S. P. XV method, except that 0.01 N potassium hydroxide was used as the standard base. Iodine numbers were determined by the method of Yasuda (5), except that 0.2 N pyridine sulfate dihydromide solution was used as the halogenating agent.

Effect of Extent of Extraction on Composition.—It was desirable to determine whether the extracted lipids were essentially those being produced by the sebaceous gland. For this reason lipids from the arms of four subjects were extracted in the usual manner. After a two-hour interval, during which time great care was taken to avoid contamination of the hands or arms, the arms were again immersed in a new supply of ether for two and one-half minutes. The extracts were then analyzed chemically and by infrared.

For another series of experiments, the arms of an individual were extracted by the standard procedure at two-day intervals for a period of six days. The material collected was then subjected to infrared analysis and acid number determination.

Lipids from the Arm and the Back.—In order to determine whether the lipid samples obtained from the arm were similar to the skin lipids from other parts of the body, both spectrophotometric and chemical comparison of the lipids from the arm were made with lipid samples from the back. Since extraction of lipids from the back by immersion was not possible, a modified procedure was followed.

The back of each subject was washed and dried in the usual manner. The subjects were then placed in a prone position, and glass cylinders 3.8 cm in diameter, 10 cm. high, and open at both ends were then

held firmly against the skin of the back. About 75 ml. of ether was then placed in the cylinder and the cylinder was carefully moved over the skin surface. The time of exposure to the solvent for each area was approximately fifteen seconds. After the extraction period, the lipid solution was removed from the cylinder with a pipet. The lipids were then recovered in the previously described manner. Extraction from the arms of the subject from the elbows to the shoulders was made by the same procedure. The gross composition of the two lipid samples was determined in the usual manner.

Deterioration During Storage.—To determine the effects of storage on skin lipids, two portions of a sample of lipids were stored under different conditions, one in a refrigerator (5–10°), and the other in a Dewar flask filled with dry ice (–80°). Chemical and spectral analyses were performed at the end of six and fifteen weeks.

Chemical Composition of Skin Lipids.—Although considerable research effort has already been directed toward the determination of skin lipid composition by others (6), it was necessary to establish the chemical nature of the component or components responsible for the observed variations in lipid composition. A liquid-liquid countercurrent extraction technique (7, 8) was therefore employed to fractionate the skin lipids.

Approximately one gram of the lipids was dissolved in petroleum ether (b. p. 35–38°) and subjected to 100 transfer stages in an all-glass, Craig-type extractor (10/10). Petroleum ether was used as the moving phase and methanol containing 2 1/2% (v/v) water as the stationary phase. After the 100th transfer, the contents of each tube were transferred to a tared aluminum moisture pan and allowed to evaporate to dryness. The solid content of each pan was then determined and infrared analyses were done on fractions of interest.

RESULTS AND DISCUSSION

Collection of Skin Lipids.—By using the described extraction procedure, it was possible to make extractions from the arms of approximately 60 to 70 people in three hours. The yield averaged 0.1 Gm. per person, a single mass extraction thus supplied about 6–7 Gm. of material. In all, over 100 Gm. of skin lipids were collected. The lipids were light brown in color and had a semisolid consistency at room temperature.

Seasonal Variation in Lipid Composition.—Infrared analysis of each of the lots of lipids indicates a possible seasonal variation in composition. Infrared spectra of typical lipid lots are shown in Figs. 1–3. The tracings are of little value in determining the absolute elemental composition of the gross mixture, however, they are useful both in establishing the relative constancy of each mixture and in detecting large variations in the amounts of different functional groups present.

Close examination of a large number of these tracings revealed that although successive samples collected over a relatively short period of time (one to two months) were quite similar, there occurred throughout the year a considerable variation in the relative amounts of the materials causing the 5.75-micron and the 5.85-micron absorption peaks. In certain lots the absorbance at 5.75 microns was greater, in others the two absorbances were about

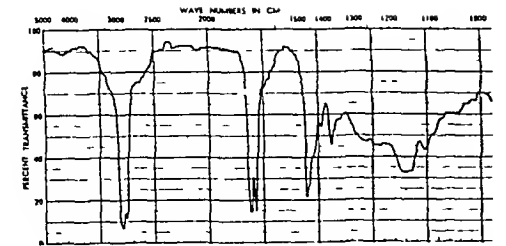


Fig. 1.—Typical infrared spectrum of lipids collected in February, March, and April, 1955.

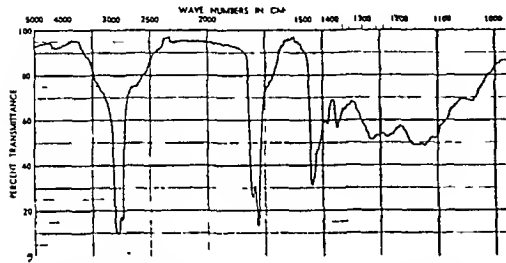


Fig. 2.—Typical infrared spectrum of lipids collected in August, September, and October, 1955.

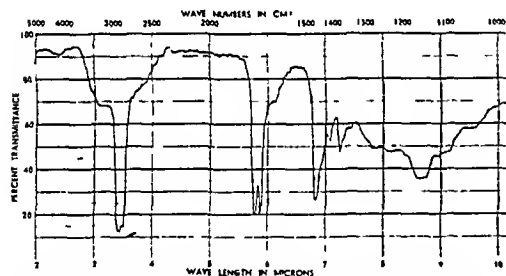


Fig. 3.—Typical infrared spectrum of lipids collected in January and February, 1956.

equal; in still others the absorbance at 5.85 microns was greater.

These variations are apparent in the data shown in Table I where the ratios of the absorbances $A_{5.75}/A_{5.85}$ for 25 different lots of skin lipids ranging from a low of 0.62 to 1.06 are given. The seasonal dependency of this ratio as indicated by the table is more apparent in Fig. 4 where it has been plotted against the time of collection.

The data suggest that the relative concentrations of the substances causing the 5.75-micron peak reach a maximum during late winter and early spring and a minimum during the months of August and September—the 5.85-micron substance following a converse pattern. This pattern of annual rise and fall is manifested during both years encompassed in the present study. This biochemical response may possibly be due to changes in the thermal environment, the diet of the volunteers during the course of the year, the presence of lipases elaborated by microorganisms, or a combination of these factors.

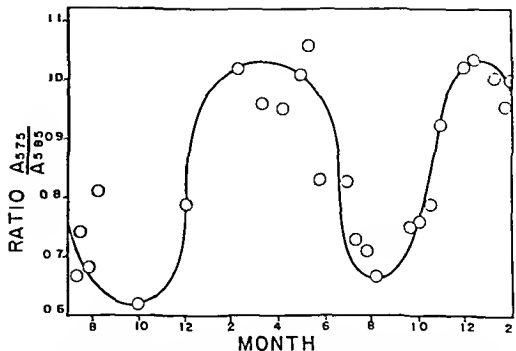


Fig. 4.—A plot showing the seasonal dependency of the absorbance ratio of the 5.75 micron to the 5.85 micron peak in the infrared (lipids from mass collections). See Table I

Since each sample represented total lipids collected from about 50 subjects, it was believed that the effects of variations among individuals were not reflected in the data shown. This assumption was verified by work done later on individuals. Results of that study will be presented in a subsequent paper.

Acid numbers obtained for some of the lipid samples are presented in Table I. Analyses were not made on every sample, since some lots were collected specifically for other studies. In this table the acid numbers of the lipid samples subjected to analysis appear to vary with the infrared absorbance

TABLE I.—RESULTS OF ANALYSIS OF MASS EXTRACT-
TIONS

Date	Infrared Peak Ratio, $A_{5.75}/A_{5.85}$	Acid No.	Iodine No.
7/6/54			
7/10	0.67		
7/16	0.74		52
7/28	0.68	73	60
8/9	0.81	78	54
10/1	0.62	109	54
12/1	0.79	61	60
2/9/55	1.02	49	68
3/9	0.96	56	60
4/6	0.95	62	69
4/28	1.01
5/11	1.06
5/25	0.83
6/29	0.83	77	50
7/13	0.73	88	42
7/27	0.71	98	43
8/10	0.67	82	52
9/21	0.75
10/5	0.76
10/19	0.79
11/2	0.92
11/30	1.02
12/14	1.03
1/11/56	1.00
1/25	0.95
2/8	1.00

ratio discussed above. This is more evident in Fig. 5 where the acid number has been plotted against the reciprocal of the ratio. The data suggest that the component or components responsible

mass extraction contained the pooled lipids of approximately 50 subjects.

Solvent.—Ethyl ether (reagent grade), which was distilled just before use to avoid the dangers associated with peroxide formation, was used as the extracting medium.

Collection Procedure.—The hands and arms of each subject were washed with a sodium lauryl sulfate solution, rinsed with distilled water, and dried. The hands were then immersed in ether for thirty seconds to insure complete removal of contaminants from this area. This portion of the ether wash was discarded.

The hand and arm were then immersed in the purified ether (1,750 ml) contained in a glass tube 100 mm. in diameter and 56 cm. high. A wooden block was used to hold the extraction tube in place. The arm was maintained in the tube for one minute; the procedure was then repeated on the other arm. The ether in the tube was replaced after 15 subjects had been subjected to the extraction procedure.

The lipids were recovered from the ether solution by distilling off the solvent. When the volume was sufficiently reduced (150 ml), the concentrated lipid solution was filtered through a medium-porosity, sintered glass filter. The clear filtrate was then carefully heated, with agitation, under a stream of nitrogen to remove all traces of solvent.

Analysis of the Lipids.—The gross chemical composition of each sample of collected skin lipids was determined by infrared analysis and by acid and iodine number determinations. Infrared spectra were obtained on a Baird Associates infrared spectrophotometer, employing a sodium chloride prism and carbon tetrachloride as the solvent. Acid numbers were determined by the U. S. P. XV method, except that 0.01 *N* potassium hydroxide was used as the standard base. Iodine numbers were determined by the method of Yasuda (5), except that 0.2 *N* pyridine sulfate dibromide solution was used as the halogenating agent.

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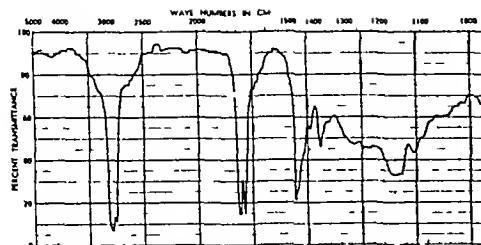


Fig. 1.—Typical infrared spectrum of lipids collected in February, March, and April, 1955.

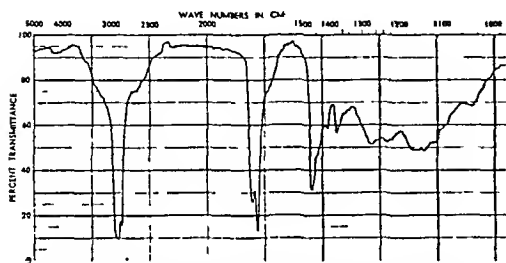


Fig. 2.—Typical infrared spectrum of lipids collected in August, September, and October, 1955.

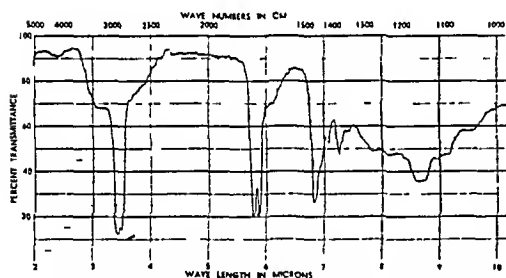


Fig. 3.—Typical infrared spectrum of lipids collected in January and February, 1956.

equal; in still others the absorbance at 5.85 microns was greater.

These variations are apparent in the data shown in Table I where the ratios of the absorbances $A_{5.75}/A_{5.85}$ for 25 different lots of skin lipids ranging from a low of 0.62 to 1.06 are given. The seasonal dependency of this ratio as indicated by the table is more apparent in Fig. 4 where it has been plotted against the time of collection.

The data suggest that the relative concentrations of the substances causing the 5.75-micron peak reach a maximum during late winter and early spring and a minimum during the months of August and September—the 5.85-micron substance following a converse pattern. This pattern of annual rise and fall is manifested during both years encompassed in the present study. This biochemical response may possibly be due to changes in the thermal environment, the diet of the volunteers during the course of the year, the presence of lipases elaborated by microorganisms, or a combination of these factors.

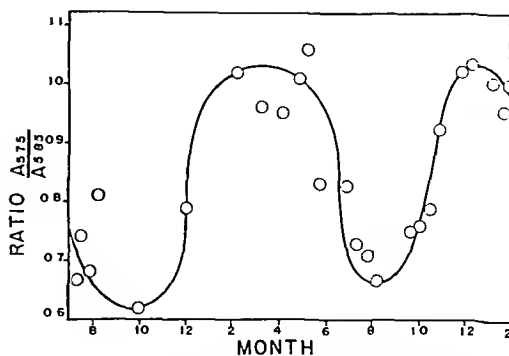


Fig. 4.—A plot showing the seasonal dependency of the absorbance ratio of the 5.75 micron to the 5.85 micron peak in the infrared (lipids from mass collections). See Table I

Since each sample represented total lipids collected from about 50 subjects, it was believed that the effects of variations among individuals were not reflected in the data shown. This assumption was verified by work done later on individuals. Results of that study will be presented in a subsequent paper.

Acid numbers obtained for some of the lipid samples are presented in Table I. Analyses were not made on every sample, since some lots were collected specifically for other studies. In this table the acid numbers of the lipid samples subjected to analysis appear to vary with the infrared absorbance

TABLE I.—RESULTS OF ANALYSIS OF MASS EXTRACTS

Date	Infrared Peak Ratio, $A_{5.75}/A_{5.85}$	Acid No	Iodine No
7/6/54			
7/10	0.67		
7/16	0.74	73	52
7/28	0.68	93	60
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10/1	0.62	109	54
12/1	0.79	61	60
2/9/55	1.02	49	68
3/9	0.96	56	60
4/6	0.95	62	69
4/28	1.01
5/11	1.06
5/25	0.83
6/29	0.83	77	50
7/13	0.73	88	42
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8/10	0.67	82	52
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11/2	0.92
11/30	1.02
12/14	1.03
1/11/56	1.00
1/25	0.95
2/8	1.00

ratio discussed above. This is more evident in Fig. 5 where the acid number has been plotted against the reciprocal of the ratio. The data suggest that the component or components responsible

for the 5.85-micron absorption are probably acidic in nature. The apparent discrepancies in the plot are probably due to the fact that a change in the amounts of the substances causing the 5.75-micron absorption peak would affect the ratio much more than it would affect the acid number.

The iodine numbers for some of the lipid samples are presented in Table I. These values, determined primarily as a check on major changes in composition, agree satisfactorily with those previously reported for skin lipids (9).

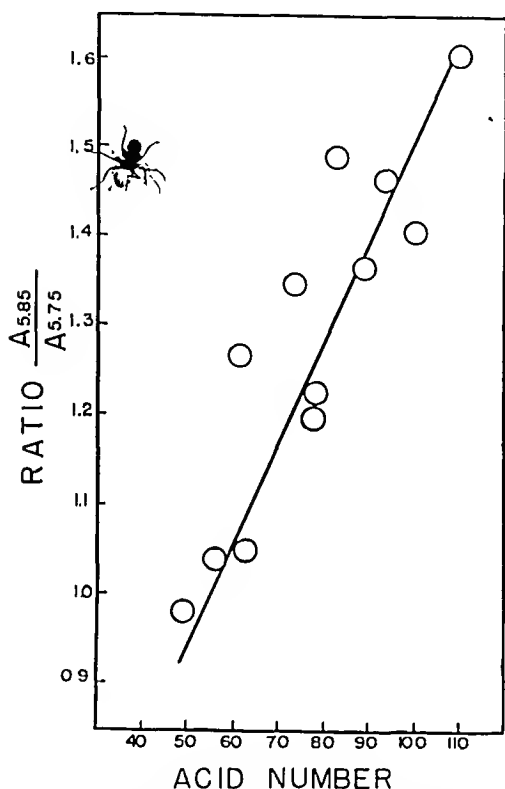


Fig. 5.—A plot showing the dependency of acid numbers on the absorbance ratio. See Table I.

Effect of Extent of Extraction on Composition.—Results of analyses on the material obtained from extractions performed at a two-hour interval are given in Table II. The data suggest that very little difference exists between the two lots.

TABLE II.—EFFECT OF SUCCESSIVE EXTRACTIONS ON COMPOSITION

Sample	Wt., Gm.	Iodine No.	Acid No.	Infrared Ratio of Absorbance, $A_{5.75}/A_{5.85}$	
First Extract	0.400	76	49	1.12	1.10 ^a
Second Extract	0.020	70	59	1.09	1.07 ^a

^a These are the results for duplicate extractions on the same individuals.

During the two-hour interval it was noted that the arms, chalky white in appearance immediately after the first extraction, became flecked with brown at the entrances to the follicular canals. This was apparently due to lipids seeping out of the canal in the normal process of resupplying the skin with the lipid surface film.

When extractions were made from one individual every two days, neither the amount nor the apparent chemical composition of the material was significantly altered by the multiple extraction procedure (see Table III). This strongly suggests that the skin lipids are fully regenerated in 48 hours at least. It would also appear, especially in view of the fact that the lipids were observed seeping out of the follicular canals, that the lipids collected are composed mainly of those secreted by the sebaceous glands.

TABLE III.—DATA SHOWING UNIFORM REGENERATION OF SKIN LIPIDS

	Initial	48 Hr.	96 Hr.	144 Hr.
Weight, mg	135	150	118	155
Acid No.	50	46	46	42
Infrared Absorbance Ratio, $A_{5.75}/A_{5.85}$	1.25	1.04	1.07	1.20

Comparison of Lipids from the Arm and the Back.—The extraction time for the area of the back in this experiment was limited to fifteen seconds due to manipulative difficulties encountered and also because the subject became too uncomfortable if longer periods of exposure were used. Then, to keep the procedures consistent, extractions from the arms were made in the same manner. The analytical results shown in Table IV indicate that arm and back lipids are quite similar.

TABLE IV.—COMPARISON OF BACK AND ARM SKIN LIPIDS

	Arms (1)	Back (1)	Arms (2)	Back (2)
Acid No.	39	39	a	a
Iodine No.	75	87	a	a
Infrared Absorbance Ratio, $A_{5.75}/A_{5.85}$	1.54	1.34	1.65	1.85

^a Amount of material collected here was too small for chemical analysis

Deterioration During Storage.—From the experimental data obtained, it is apparent that the extracted lipids can be stored for extended periods without undergoing significant chemical changes. Results of the various analyses are given in Table V.

The initial iodine number is somewhat lower than the others, but no significant differences exist between the stored lots and the original sample. It thus appears that the lipids can be stored for long periods of time if kept at cold temperatures.

TABLE V.—DATA SHOWING EFFECTS OF STORAGE ON SKIN LIPIDS

	Original	Storage Time			
		6 Weeks Dry Ice	Refrig.	15 Weeks Dry Ice	Refrig.
Acid Number	56	56	56	56	56
Iodine Number	60	71	70	69	71
Infrared Absorbance Ratio, $A_{5.75}/A_{5.85}$	0.94	0.98	0.98	1.01	1.00

Chemical Composition of Skin Lipids.—In Fig. 6 a typical distribution pattern obtained by the countercurrent extraction procedure is shown. It was evident from infrared analyses that nearly complete separation of the materials causing the two absorption peaks was accomplished.

The material found in Tubes 40–42 was crystalline in appearance, strongly absorbed infrared light in the 5.85-micron region, and had an acid number of 180. Further countercurrent extractions were performed on this fraction in an effort to purify it. Although only one peak continued to appear in the chromatograms, successive extractions did help to purify the material. Infrared tracings of the purified material indicated that it was composed largely of fatty acids of the palmitic, stearic, and arachidic type. A neutral equivalent determination resulted in an equivalent weight of 306, which is in the range of the mixtures of C_{16} to C_{20} fatty acids previously reported (10). Furthermore, melting point determinations on various fractions resulted in melting points ranging from 50 to 60°, which is also in the range of mixtures of the above acids. None of these fractions gave a positive Lieberman-Burchard test for sterols.

The material in Tube 95 of the countercurrent transfer operation did not appear crystalline, strongly absorbed infrared light in the 5.75-micron region, and had an acid number of only five. Infrared tracings indicated that esters were the major components of this fraction. This was subsequently substantiated by saponification.

About 0.5 Gm. of the material believed to contain esters was refluxed with 20% alcoholic potassium hydroxide for nine hours. After extraction and purification, the nonsaponifiable portion did not absorb in the 5.75-micron region; while the 5.85-

micron peak (indicating carboxylic acids), which was absent before saponification, appeared in the infrared spectrum of the base-soluble portion of the mixture. This definitely showed that esters were responsible for the 5.75-micron absorption peak.

The material in Tube 95 of the original countercurrent transfer operation gave a positive Lieberman-Burchard test, indicating that sterols were also present in this fraction. More work is currently being done in an effort to further fractionate the nonacid portion of the skin lipids.

These data indicate that the chemical species responsible for the observed seasonal variations in human skin lipids are free fatty acids and esters of fatty acids, since it has now been definitely established that these chemical entities are responsible for the observed absorbance peaks at 5.85 and 5.75 microns, respectively, in the infrared tracings.

SUMMARY

A procedure for extracting relatively large quantities of human skin lipids is presented. The lipids collected were found to vary significantly with the season of the year. Successive extractions within a short time (two hours and also two days) did not alter the composition of the lipids, nor did extractions performed every two days alter the amount of lipids collected. The lipids from the arms were found to be quite similar to those obtained from the back. The lipids were found to be quite stable if stored at temperatures below 10°. A countercurrent extraction procedure for the fractionation of skin lipids is presented. Fatty carboxylic acids and esters of those acids, which absorb in the 5.85 and 5.75-micron regions, respectively, of the infrared spectrum, are deemed responsible for the observed seasonal variations in human skin lipid composition.

REFERENCES

- (1) Rothman, S., *J. Lob. Clin. Med.*, 28, 1305(1943).
- (2) MacKee, G. M., *et al.*, *Science*, 98, 66(1943).
- (3) MacKee, G. M., *et al.*, *J. Invest. Dermatol.*, 6, 43(1945).
- (4) Summary of Discussion, Conference of Jan. 28, 1957, Dermatological Research Lab., Mass. Gen. Hosp., Boston Mass.
- (5) Yasuda, M., *J. Biol. Chem.*, 94, No. 2 (1931).
- (6) Rothman, S., "Physiology and Biochemistry of the Skin," Chapter 13, University of Chicago Press (1954).
- (7) Perry, E. S., and Brokaw, C. Y., *J. Am. Oil Chemists' Soc.*, 32, 191(1955).
- (8) Blankenhorn, D. H., and Ahrens, E. H., Jr., *J. Biol. Chem.*, 212, 69(1955).
- (9) Ricketts, C. R., *et al.*, *Clin. Sci.*, 10, 89(1951).
- (10) Rothman, S., "Physiology and Biochemistry of the Skin," 317, University of Chicago Press (1954).

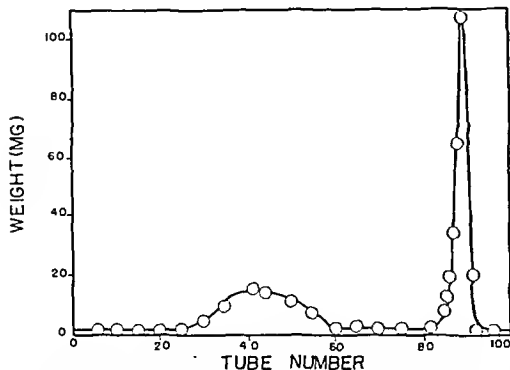


Fig. 6.—A plot showing the distribution of human skin lipids between petroleum ether and 97.5% methanol (100 transfers).

Investigation of Human Skin Lipids II*

By JAMES E. TINGSTAD, DALE E. WURSTER, and TAKERU HIGUCHI

Skin lipids from different individuals vary considerably in chemical composition. The seasonal variation in skin lipid composition suggested in a previous report has been confirmed. The nonsaponifiable fraction of skin lipids can be further fractionated using a liquid-liquid countercurrent extraction procedure.

IN A RECENT REPORT from these laboratories (1) a possible seasonal variation in the chemical composition of human skin lipids was suggested. However, since the results were obtained on samples containing the pooled lipids of a large number of persons, the possibility existed that the observed variations were artifacts resulting from variations among the skin lipids of the various individuals from whom the lipids were obtained. Therefore, this study was initiated in an attempt to verify the seasonal variation phenomenon and to ascertain the differences in skin lipid composition among individuals.

It was previously reported (1) that skin lipids could be fractionated using a liquid-liquid countercurrent extraction procedure. This resulted in an acid and a nonacid portion, the latter consisting mostly of wax alcohols, sterols, esters, and hydrocarbons. The saponification of the esters produced free fatty acids and a nonsaponifiable fraction consisting mainly of alcohols and hydrocarbons. In this report, results of an attempt to further fractionate this nonsaponifiable fraction are presented

EXPERIMENTAL

Variations in Skin Lipid Composition Among Individuals.—The arm lipids from ten individuals were obtained in the manner previously described (1). Extractions were made every four weeks for a period of fifteen months. The lipid samples obtained from each individual were treated separately. The gross composition of each sample was followed with infrared analysis and acid number determinations.

Fractionation of the Nonsaponifiable Fraction of Skin Lipids.—A sample (400 mg.) of the nonsaponifiable fraction of the skin lipid material was dissolved in petroleum ether (b. p. 35–38°) and subjected to 100 transfer stages in an all-glass, Craig-type extractor (10/10). Petroleum ether was used as the moving phase and methanol containing 2½% (v/v) water as the stationary phase. After the 100th transfer, the contents of each tube were transferred to a tared aluminum moisture pan and allowed to evaporate to dryness. The solid content

of each pan was then determined and infrared analyses were performed on the fractions of interest.

RESULTS AND DISCUSSION

Seasonal Variations in Skin Lipid Composition Among Individuals.—As previously reported (1) the absorbance ratio, $A_{5.75}/A_{5.85}$, obtained from infrared studies on pooled human skin lipids, where the 5.75-micron peak represents esters and the 5.85-micron peak represents carboxylic acids, showed a marked seasonal variation. The absorbance ratios obtained for the ten individuals used in this study show the same periodic fluctuation. Results of the infrared analyses and acid number determinations of lipid samples from two individuals representing the extremes of the test group are given in Table 1.

TABLE 1.—RESULTS OF ANALYSIS OF INDIVIDUAL LIPID EXTRACTS OBTAINED FROM SUBJECTS REPRESENTING EXTREMES OF THE TEST GROUP

Date	Sample, Test Subject No.	Infrared Peak Ratio, $A_{5.75}/A_{5.85}$	Acid Number
1/10/56	3	2.03	..
2/22	3	1.19	40
3/19	3	1.42	31
4/16	3	1.65	33
5/14	3	1.23	51
6/11	3	1.25	44
7/9	3	1.02	48
8/6	3	1.06	60
9/6	3	1.14	68
10/4	3	1.48	39
10/30	3	1.24	41
11/27	3	1.62	36
1/9/57	3	1.75	32
2/8	3	1.55	34
1/10/56	6
2/22	6	0.87	54
3/19	6	0.70	50
4/16	6	0.64	57
5/14	6	0.58	52
6/11	6	0.47	72
7/9	6	0.39	73
8/6	6	0.54	64
9/6	6	0.46	66
10/4	6	0.54	58
10/30	6	0.57	59
11/27	6	0.77	57
1/9/57	6	1.03	40
2/8	6	0.81	45

* Received July 11, 1957, from the laboratories of the School of Pharmacy, University of Wisconsin, Madison.

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Based on a dissertation submitted by James E. Tingstad to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

It was immediately evident from the collected data that great variations in lipid composition exist among individuals; however, even though these differences do exist, the lipids of each tested subject

followed the same general pattern as far as the seasonal variation is concerned. This is particularly evident when the data is plotted as in Fig 1. Here it can be observed that the peaks and valleys in this plot occur at the same time of the year as they did in a similar plot for the lipids collected in the previously reported mass extractions (1). The same phenomenon was observed in all the members of the test group. This strongly indicates that a seasonal variation in the skin lipid composition of individuals does occur in this climate.

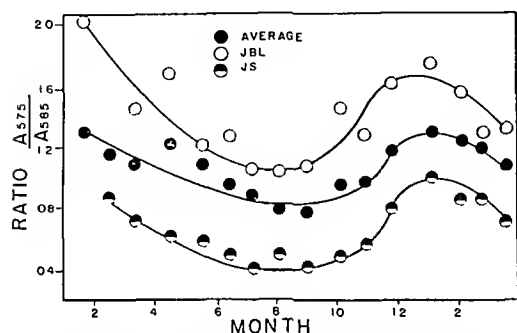


Fig 1—A plot showing the seasonal dependency of the absorbance ratio of the 5.75-micron (esters) to the 5.85 micron peak (carboxylic acids) in the infrared (● Average for the 10 individuals tested, ○ JBL—test subject no 3, ◐ JS—test subject no 6. See Table I.)

Fraction of the Nonsaponifiable Fraction of Skin Lipids.—The separation of the nonsaponifiable fraction of skin lipids into two fractions is shown in Fig 2. Infrared tracings showed that the first fraction contained appreciable amounts of alcohols and the second fraction contained mostly hydrocarbons. This was further indicated by the fact

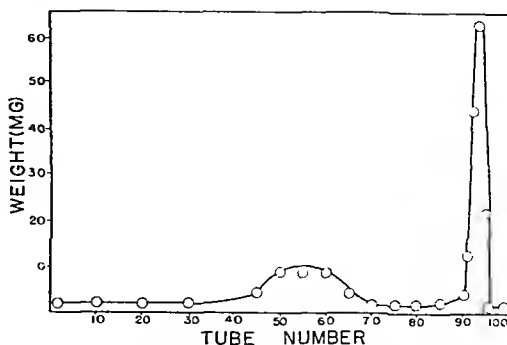


Fig 2—A plot showing the distribution of the nonsaponifiable fraction of human skin lipids between petroleum ether and 97.5% methanol (100 transfers)

that the first fraction gave a positive Lieberman Burchard test for sterols, while the second did not. Thus it appears that the liquid-liquid counter-current extraction procedure is a suitable method for the fractionation of human skin lipids.

SUMMARY

A considerable variation in the chemical composition of skin lipids exists among individuals. A seasonal variation in skin lipid composition, which appears to be common to most people in this climate, was demonstrated in ten individuals. A procedure for the fractionation of the nonsaponifiable fraction of human skin lipids is presented.

REFERENCE

- (1) Tingstad J. E., Wurster, D. E. and Higuchi T. *THIS JOURNAL* 47, 188(1958)

A Bacteriological Study of Ophthalmic Ointments*

By RAYMOND W. VANDER WYK† and ANDREW E. GRANSTON

A method was devised to determine the number of bacteria present in a gram of ophthalmic ointment. Eighty-three commercial ophthalmic ointments were tested for contamination by bacteria using this method. Seventy-one of these ointments contained bacteria. Benzyl alcohol was incorporated into several ophthalmic ointments as an antibacterial agent. The benzyl alcohol greatly reduced the number of bacteria present.

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IN RECENT YEARS considerable attention has been directed toward the desirability of producing and maintaining the sterility of liquid ophthalmic preparations (1). Several workers have described methods for sterilization including the use of heat (2), the use of chemical antibacterial agents (3), and the use of bacterial excluding filters (4).

However, a survey of the literature has revealed that very little work has been published concerning the testing for sterility of ophthalmic ointments. Some research was done by Lehrfeld and Donnelly (5), who tested for the most part the sterility of partly used tubes of ointment.

Since no serious study had been previously undertaken to determine the sterility of unused tubes of ophthalmic ointments, the purpose of this research was to examine bacteriologically a representative number of commercially available ophthalmic ointments; and also to investigate the possibility of producing and maintaining the sterility of ophthalmic ointments by the incorporation of antibacterial agents.

EXPERIMENTAL

Preparation.—Into a 250-ml. Erlenmeyer flask was poured 25.0 ml. of distilled water and a small number of 0.5-mm. glass beads. Then the flask was plugged and sterilized in an autoclave.

Testing.—Each ointment to be tested was immersed for one hour in its unopened tube in a 1:1,000 solution of benzalkonium chloride in order to kill any bacteria present on the surface of the tube.

The tube was removed from the benzalkonium chloride solution, the lip of the flask and the tip of the tube were flamed simultaneously, and the contents of the tube were squeezed into the flask.

Then the flask was warmed in a constant-temperature water bath to 45°. At this temperature the ointment melted and was easily dispersed throughout the water by being shaken on an agitator for one hour at room temperature.

At the end of the agitation period, three 1.0-ml. portions were transferred with a sterile pipet into three sterile Petri dishes. To these was added melted blood agar in the usual manner for preparing pour plates. After mixture by gentle rotation and after solidification of the agar, the plates were incubated for twenty-four hours at 37°.

Determination of the Number of Bacteria per Gram.—The number of bacteria in 1 Gm. of each sample was determined by counting the colonies on the plate. Each colony was assumed to represent one organism in the ointment. The following formula was used in this determination:

$$\begin{aligned} \text{No. of bacteria per Gm.} = \\ (\text{No. of colonies per plate} \times 25) / \\ (\text{Wt. of contents of the tube}) \end{aligned}$$

In all instances the weight of the contents of the tube was 3.5 Gm.

Results.—Of the 83 commercially available ophthalmic ointments tested 12, or 14.5%, were sterile. However, only 19 of the 71 nonsterile ointments had 50 or more organisms per gram.

For convenience in discussing the results of this work the ointments have been classified according to their principal chemotherapeutic agents.

Antibiotic Ointments.—Twenty-eight were tested and only eleven were sterile. However, the bacterial count was low, the average bacterial count being only 12 organisms per gram. The results appear in Tables I, II, and III.

Sulfa-Containing Ointments.—Nine were tested, and only one was sterile. However, the average bacterial count was only 15 organisms per gram. The results are shown in Table IV.

Mercury-Containing Ointments.—Twenty-one ointments were tested, and none was found to be

TABLE I.—PENICILLIN OPHTHALMIC OINTMENTS

Name of Ointment	Bacteria/ Gm.
Crystalline procaine penicillin G, 5,000 units/Gm.	14
Penicillin calcium, 100,000 units/Gm.	0
Penicillin G, 1,000 units/Gm.	
Sample 1	7
Sample 2	14
Penicillin G, 100,000 units/Gm.	7
Penicillin G potassium, 1,000 units/Gm.	0
Procaine penicillin G, 1,000 units/Gm.	64

TABLE II.—ANTIMOTIC OINTMENTS CONTAINING CORTISONE OR HYDROCORTISONE

Name of Ointment	Bacteria/ Gm.
Cortisone acetate 1.5% with bacitracin, 1,000 units/Gm.	14
Hydrocortisone acetate 0.5% with neomycin sulfate, 5 mg./Gm.	14

TABLE III.—ANTIMOTIC OINTMENTS CONTAINING ANTIBIOTICS OTHER THAN PENICILLIN

Name of Ointment	Bacteria/ Gm.
Bacitracin, 500 units/Gm.	
Sample 1	0
Sample 2	0
Sample 3	7
Sample 4	7
Sample 5	0
Chloramphenicol, 1%	0
Chlortetracycline hydrochloride, 1%	0
Erythromycin, 5 mg./Gm.	
Sample 1	129
Sample 2	7
Neomycin sulfate, 5%	
Sample 1	7
Sample 2	0
Neomycin hydrochloride, 2.5 mg./Gm., and gramicidin, 0.25 mg./Gm.	7
Neomycin sulfate, 5 mg./Gm., polymyxin B sulfate, 5,000 units/Gm., and bacitracin, 400 units/Gm.	
Sample 1	0
Sample 2	14
Oxytetracycline hydrochloride, 5 mg./Gm., with polymyxin B sulfate, 10,000 units/Gm.	0
Polymyxin B sulfate, 20,000 units/Gm.	0
Tetracycline hydrochloride, 1%	
Sample 1	7
Sample 2	14
Sample 3	7

sterile. The average number of bacteria per gram for these ointments was 44. The results are shown in Table V.

Cortisone and Hydrocortisone Ointments.—Four ointments were tested and none was sterile. All showed identical bacterial counts of 14 bacteria per gram. This low count is very surprising in view of the fact that none of the ointments tested contained anti-infective agents. The results appear in Table VI.

Boric Acid Ointments.—Six were tested and none was found to be sterile. The average number of

TABLE IV.—OPHTHALMIC OINTMENTS CONTAINING SULFA DRUGS

Name of Ointment	Bacteria/ Gm.
Sodium sulfacetamide, 10%	14
Sulfadiazine, 5%	
Sample 1	7
Sample 2	21
Sulfanilamide, 5%, phenacaine hydrochloride, 1%, and cod liver oil concentrate	21
Sulfathiazole, 5%	
Sample 1	7
Sample 2	0
Sulfathiazole, 5%, and phenacaine hydrochloride, 2%	7
Sulfathiazole, 5%, and piperocaine hydrochloride, 4%	36
Sulfisoxazole diethanolamine, 4%	21

TABLE V.—OPHTHALMIC OINTMENTS CONTAINING MERCURY COMPOUNDS

Name of Ointment	Bacteria/ Gm.
Ammoniated mercury, 3%	107
Butacaine sulfate, 2%, and nitromersol, 1:3,000	29
Mercuric chloride, 1:3,333	14
Mercuric chloride, 1:5,000	
Sample 1	21
Sample 2	50
Phenacaine hydrochloride, 2%, with mercurbolide	21
Phenylmercuric nitrate, 1:30,000	14
Phenylmercuric nitrate, 1:20,000, and phenacaine hydrochloride, 1%	36
Piperocaine hydrochloride, 4%, and thiomersal, 1:5,000	36
Thiomersal, 1:5,000	64
Yellow mercuric oxide, 1%	
Sample 1	64
Sample 2	14
Sample 3	50
Sample 4	57
Sample 5	43
Yellow mercuric oxide, 2%	
Sample 1	14
Sample 2	121
Sample 3	43
Sample 4	36
Sample 5	50
Sample 6	36

TABLE VI.—OPHTHALMIC OINTMENTS CONTAINING CORTISONE OR HYDROCORTISONE

Name of Ointment	Bacteria/Gm.
Cortisone acetate, 1.5%	
Sample 1	14
Sample 2	14
Hydrocortisone acetate, 1.5%	
Sample 1	14
Sample 2	14

Fifteen ointments were tested. As might be expected in the absence of antibacterial agents, this group showed the highest bacterial counts. The average bacterial count for this group of ointments was 65. The results appear in Table VIII.

TABLE VII.—OPHTHALMIC OINTMENTS CONTAINING BORIC ACID

Name of Ointment	Bacteria/Gm.
Boric acid, 5%	
Sample 1	79
Sample 2	21
Sample 3	57
Sample 4	14
Boric acid, 10%	
Sample 1	136
Sample 2	21

TABLE VIII.—MISCELLANEOUS OPHTHALMIC OINTMENTS

Name of Ointment	Bacteria/ Gm.
Atropine sulfate, 1%	
Sample 1	21
Sample 2	129
Butacaine sulfate, 1%	7
Butacaine sulfate, 2%	7
Cod liver oil concentrate	43
Phenacaine hydrochloride, 1%	
Sample 1	36
Sample 2	50
Phenacaine hydrochloride, 1%, and ephedrine hydrochloride, 0.4%	157
Phenacaine hydrochloride, 1%, and epinephrine, 1:50,000	
Sample 1	36
Sample 2	243
Sample 3	14
Physostigmine salicylate, 0.25%	43
Piperocaine hydrochloride, 4%	107
Thenylpyramine, 0.5%	36
Zinc sulfate, 0.5%	43

OPHTHALMIC OINTMENTS CONTAINING ANTIBACTERIAL AGENTS

A Specially Formulated Ointment Base.—With few exceptions the commercial samples of ophthalmic ointments previously tested contained petrolatum in their formulas. Since petrolatum is not the ideal ointment base for topical application, a different base was decided upon. This base has the following formula:

White Wax.....	1 part
Cetyl Alcohol.....	1 part
Delyl Extra ¹	4 parts

The white wax and the cetyl alcohol were melted in a beaker and the warm Delyl Extra was added. The mixture was stirred until it congealed.

The ointment base contains 0.16% of water and is miscible with the the lachrymal secretions. It can be sterilized by dry heat for two hours at 175° without apparent breakdown.

¹ Delyl Extra is manufactured by Givaudan Delawanna, Inc.

bacteria per gram was 55. The results are shown in Table VII.

Miscellaneous Ointments.—This group consisted of ophthalmic ointments, with the exception of cortisone, which are used for purposes other than anti-infective.

A Method for Testing the Effectiveness of Added Antibacterial Agents.—Twenty grams of ointment base were prepared and sterilized. Four 4-mm. loopfuls of a twenty-four-hour nutrient broth culture of *Micrococcus pyogenes* var. *aureus* were added and mixed thoroughly. Three and one-half grams of this contaminated base were placed in a sterile ophthalmic tube as a control.

To 9.5 Gm. of the remaining base was added 0.5 ml. of a 1:250 dilution of benzalkonium chloride. The final concentration of benzalkonium chloride was 1:5,000. The mixture was tubed in 3.5-Gm. amounts.

Within eight hours of preparation, each tube was tested to determine the number of bacteria per gram. The method is described under "Experimental."

A similar study was made with final concentrations of 0.5% chlorobutanol, 0.5% benzyl alcohol, and a mixture of 0.25% chlorobutanol and 0.25% benzyl alcohol.

In considering the effectiveness of the antibacterial agents used, it should be borne in mind that the number of organisms added to each ointment was far in excess of the number normally found in any commercial ointment. This was done purposely in order to obtain results which would be more significant. The effectiveness of each agent was based upon the number of organisms killed or inhibited as compared to a control. The results are shown in Table IX.

TABLE IX.—A COMPARISON OF THE BACTERICIDAL EFFECTIVENESS OF CERTAIN ANTIBACTERIAL AGENTS IN A CONTAMINATED OINTMENT BASE

Antibacterial Agent	No. of Organisms/Gm.	Killed, %
Benzalkonium chloride, 1:1000	835	98.0
Chlorobutanol, 0.5%	4,480	89.3
Benzyl alcohol, 0.5%	171	99.6
Chlorobutanol, 0.25%, and benzyl alcohol, 0.25%	11,409	72.7
None (control)	41,729	..

Another Method of Testing the Effectiveness of Added Antibacterial Agents.—Only benzyl alcohol 0.5% was used since this agent with a killing or inhibiting activity of 99.6% was the most effective antibacterial agent used in this study.

Six ophthalmic ointments with formulas similar to those of commercially available products were prepared and benzyl alcohol was added. The final concentration of benzyl alcohol was 0.5%. Each ointment was prepared as previously described.

Bacterial counts were taken on each extemporaneously prepared sample, and the results were compared with those previously obtained for similar types of commercial samples (see Tables I, V, VI, and VII). If similar ointments from more than one manufacturer had previously been tested, the average number of bacteria per gram was taken as the basis for comparison.

The names of the ointments were yellow mercuric oxide 1%, yellow mercuric oxide 2%, boric acid 5%, boric acid 10%, cortisone acetate 1.5%, and penicillin G potassium 1,000 units per gram.

Results.—The incorporation of benzyl alcohol

0.5% in extemporaneously prepared ointments resulted in a reduction of the bacterial count in all ointments. The actual reduction by the use of benzyl alcohol was 97.1%. (See Table X.)

TABLE X.—A COMPARISON BETWEEN EXTEMPORANEOUSLY PREPARED OPHTHALMIC OINTMENTS CONTAINING BENZYL ALCOHOL 0.5% AND COMMERCIAL OPHTHALMIC OINTMENTS

Name of Ointment	Extemporaneously Prepared Samples, Bacteria/Gm.	Commercial Samples, Av. No. Bacteria/Gm.
Yellow mercuric oxide, 1%	0	46
Yellow mercuric oxide, 2%	7	50
Boric acid, 5%	0	43
Boric acid, 10%	0	79
Cortisone acetate, 1.5%	0	14
Penicillin G potassium, 1000 units/Gm.	0	11

SUMMARY

- Eighty-three commercial samples of ophthalmic ointments were examined bacteriologically to determine the number of organisms per gram of each. Eighty-five and one-half per cent were nonsterile.
- A specially formulated ointment base was purposely contaminated with a culture of *Micrococcus pyogenes* var. *aureus*. To this mixture was added various antibacterial agents. These ointments were tested to determine the killing or inhibitory activity of the added antibacterial agents.
- Benzyl alcohol 0.5 per cent was 99.6 per cent effective; benzalkonium chloride 1:5000 was 98.0 per cent effective; chlorobutanol 0.5 per cent was 89.3 per cent effective; while a mixture of chlorobutanol 0.25 per cent and benzyl alcohol 0.25 per cent was only 72.7 per cent effective.

4. Six ophthalmic ointments were prepared extemporaneously by methods similar to those used by the pharmaceutical manufacturers. Benzyl alcohol 0.5 per cent was added to each. The number of organisms per gram of each ointment was determined and was compared with the average number per gram of commercially available ointments with similar formulas. Benzyl alcohol reduced the number of bacteria per gram by 97.1 per cent.

REFERENCES

(1) Theodore, P. H., and Minsky, H. J., *J. Am. Med. Assoc.*, 147, 1381 (1951).
(2) Florentin, M., *Bull. Am. Soc. Hosp. Pharmacists*, 10, 56 (1953).
(3) Kaplan, A. O., Master of Science thesis, Massachusetts College of Pharmacy, 1955.
(4) Croumey, E. F., *Am. Prof. Pharm.*, 21, 922 (1955).
(5) Lehrfeld, L., and Donnelly, E. G., *Arch. Ophthalmol.*, 40, 39 (1948).

Toxicity Studies of Sulfur Hexafluoride (SF₆) in Pneumoperitoneum*

By HAROLD C. HODGE, W. GEORGE SWALBACH†, and HERMANN RAHN‡

The results of studies of rats and dogs maintained with pneumoperitoneum of sulfur hexafluoride, and with air for control observations, for periods of six months are reported. The toxicity found was of a sufficiently low order to warrant initial treatments of humans.

EVER SINCE THE INTRODUCTION of artificial pneumothorax and pneumoperitoneum for therapeutic reasons, the reabsorption of the injected gas has been noted. For prolonged therapy this reabsorption has been a nuisance requiring refillings from time to time. It was noted early that pure oxygen disappeared much faster than air, and today air is the common gas used. Many attempts have been made during the last fifty years to find a more suitable gas than air—a gas which would be more slowly absorbed from the cavity. Webb, *et al.* (1), found no difference between air and nitrogen. Grass and Meiners (2) employed argon which was absorbed faster than air. Helium was tried unsuccessfully by Schedtler (3). More recently helium was found to extend the time between refills four to six days in pneumoperitoneum patients (4). Comparing the absorption rates of various gases from closed body pockets (5-8), however, led to the general conclusion that of all the gases so far tested nitrogen is the slowest to diffuse and therefore is most suitable for a prolonged collapse therapy. It was, therefore, of some interest when Tenney, Carpenter, and Rahn (9) showed that the inert gas SF₆ when injected into a body cavity, not only disappeared more slowly than nitrogen, but actually produced a temporary doubling of the pneumoperitoneum volume before it slowly disappeared.

Before administering SF₆ gas to pneumoperitoneum patients, it was necessary to demonstrate the lack of toxicity of this gas. This paper presents the results of studies of rats and dogs maintained with pneumoperitoneum of SF₆, and with air for control observations, for periods of six months. Based on these observations the initial treatments of patients reported elsewhere (10) were undertaken.

RAT STUDIES

Groups of young, albino rats, 20 males and 20 females per group, of the Rochester-Wistar strain were selected and matched approximately for average body weight. The rats were housed five to a cage in metal cages in which pans filled with shavings were used for bedding. The ration was Purina Fox Chow Meal, the drinking water was tap water; both were supplied *ad libitum*. As can be seen in Table I, the average initial body weight for the male rats ranged from 149 to 154 Gm., for the female rats from 128 to 132 Gm. The treatment plan is indicated in Table I: each experimental rat was in-

TABLE I

Treatment	Sex	No. of Survivors	Av. Body Weight (Gm.) Initial	Final
Air intrap.	M	8	149	412
SF ₆ intrap.	M	9	151	400
Air subcut.	M	15	154	385
SF ₆ subcut.	M	15	153	356
SF ₆ subcut.	F	18	132	245
Air subcut.	F	13	129	244
SF ₆ intrap.	F	14	128	242
Air intrap.	F	12	129	225

jected with a volume of SF₆ sufficient to distend the skin tightly; each control rat was injected with an approximately equal, or perhaps slightly larger, volume of air. The subcutaneous injections were made in the midline of the shoulder area. The injections of SF₆ or air were repeated at whatever intervals were necessary to maintain some inflation. The intervals between injections varied from one to two weeks. No precautions to maintain sterility were observed.

Body Weight.—Good weight gains were recorded by every group. In the case of the male rats, only those given subcutaneous injections of SF₆ showed a growth less than that typical of the colony norm (380 Gm. at the same age). A number of the rats had shown areas of infection in the inflated skin, which may account for the depressed growth. In the case of the female rats, only the group given air intraperitoneally grew somewhat less rapidly than the colony norm (235 Gm. in rats of the same age). The male rats given SF₆ subcutaneously showed the greatest growth retardation, whereas the female rats given SF₆ subcutaneously had the highest average weight. The deviations in the growth curves are probably coincidental; there is nothing in the growth curves to indicate a specific toxic effect of SF₆.

Mortality.—In the male rats given air or SF₆ subcutaneously, identical mortalities were observed throughout the experimental period. Negligible differences occurred when the two gases were administered intraperitoneally. The subcutaneous administration produced only 25% mortality; whereas given intraperitoneally, mortalities by the

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end of the thirty-sixth week were 60 to 65%. In the female rats, higher mortalities were observed when air was administered than when SF_6 was administered. Given subcutaneously, the accumulated mortality with air injections reached about 35%; whereas with SF_6 , the maximum was only 10% mortality. The total accumulated mortalities by the end of the period were 30 and 40%, respectively, when air and SF_6 were given intraperitoneally. No sterile precautions were used in injecting the gases. During the first month the rats did not respond adversely to the injections. After a period of about ten weeks, however, it became apparent that many rats were dying in the few days following each injection; fatal infections were suspected. Beginning at this time, penicillin was routinely administered parenterally simultaneously with the gas injections. Almost without exception, deaths no longer occurred in the days immediately following the gas injections. There is no indication in the mortality data that SF_6 is more toxic than air.

The higher mortality rates with intraperitoneal injections than with subcutaneous injections can be explained as an injection artifact. On several occasions it was observed that animals died immediately with peritoneal refills. Autopsy showed that in each case the needle had punctured a blood vessel and that large gas bubbles were present in the circulation. Such accidents never occurred when gas was given subcutaneously.

Organ Weights.—Rats were sacrificed after thirty-six weeks on the experimental regimen and gross autopsy examinations were conducted under the direction of Dr. Richard C. Crain of the Department of Pathology. A number of organs were dissected out and weighed, these included: the liver, kidney, lungs, brain, stomach, heart, and spleen. The average organ weights are given for each of the experimental groups in Table II. The average

jected, either subcutaneously or intraperitoneally.

Pathology.—Sections were prepared using hematoxylin and eosin stains of the following tissues: heart, lungs, spleen, stomach, small and large intestines, kidney, adrenal, testes or ovary, urinary bladder, bone marrow, and brain; in addition, any abnormal tissue was sampled for study. Sections of skin were removed from the shoulder area of the rats receiving subcutaneous administration of gas and sections of the abdominal wall were taken from those animals receiving gas intraperitoneally. The histological study was carried out by Dr. Robert D. Coye, Jr. of the Department of Pathology. Skin sections from three males and three female rats, given air or SF_6 intraperitoneally, and from the same number, injected subcutaneously, were also examined by Dr. D. L. Opdyke of the Procter and Gamble Company. These tissue samples were fixed in formalin solution. Routine paraffin sections were prepared and stained with hematoxylin and eosin.

No lesions were found that were attributed to a toxic effect of SF_6 . Dr. Opdyke described normal blood vessels, collagen fibers, and sebaceous glands; hair follicles revealed no vesicles or any signs of chemical or structural changes (Fig. 1). There was no hyperplasia, keratinization was normal, no evidence of infiltration, nor any indication of inflammatory reactions were seen. In a few rats, during the course of this study, areas of infection developed locally in the inflated skin above the subcutaneous injection. Presumably the pressure had reduced, at least in some measure, the circulation into the inflated areas of the skin. No lesions were found in the peritoneal tissue of the rats at the end of the study. The deaths observed during the experimental period were attributed to secondary infections. Of the 29 rats that died during the experiment, 14 were diagnosed at autopsy as dying of acute infection or of hemorrhage. These deaths

TABLE II AVERAGE ORGAN WEIGHTS

Sex	Treatment	Route	Liver	Kidneys	Testes	Lungs	Brain	Stomach	Heart	Spleen	No. Rats
M	Air	Subcut.	12.29	2.92	3.02	2.40	2.05	2.62	1.45	1.48	14 ^a
M	Air	Intrap.	13.73	3.33	3.35	2.93	2.08	2.00	1.49	1.31	8
M	SF_6	Subcut.	11.62	2.86	3.08	2.29	1.90	1.89	1.45	1.47	15
M	SF_6	Intrap.	12.53	3.11	3.19	2.13	1.99	1.78	1.30	1.29	9
F	Air	Subcut.	7.83	1.95	...	1.66	1.86	1.51	1.03	1.09	13
F	Air	Intrap.	7.93	1.98	...	1.75	1.86	1.47	0.97	1.07	12
F	SF_6	Subcut.	8.42	2.07	...	1.85	1.89	1.56	0.99	0.95	17
F	SF_6	Intrap.	8.22	2.01	...	1.66	1.86	1.52	1.02	0.94	14
Average Organ Weight/Body Weight Ratios (mg./Gm. b. wt.)											
M	Air	Subcut.	32.01	7.69	7.97	6.34	5.42	5.41	3.83	3.84	
M	Air	Intrap.	33.33	8.09	8.15	7.14	5.07	4.85	3.62	3.20	
M	SF_6	Subcut.	32.80	8.11	8.59	6.69	5.45	5.29	4.16	4.23	
M	SF_6	Intrap.	31.22	7.77	8.07	5.31	5.03	4.47	3.30	3.20	
F	Air	Subcut.	32.14	7.99	...	6.86	7.71	6.22	4.24	4.46	
F	Air	Intrap.	35.17	8.82	...	7.83	8.28	6.55	4.34	4.70	
F	SF_6	Subcut.	34.40	8.48	...	7.60	7.76	6.42	4.05	3.89	
F	SF_6	Intrap.	33.88	8.40	...	6.82	7.69	6.25	4.19	3.86	

^a Liver weights on 15 rats; brain weights on 13 rats.

organ weights calculated on the basis of body weights are also presented for each group. All of the organ weights were within the normal ranges; there was no indication of any abnormality when SF_6 was in-

were approximately equally divided between the control and the experimental groups. In many other cases, autopsy examinations were valueless because autolysis was advanced.

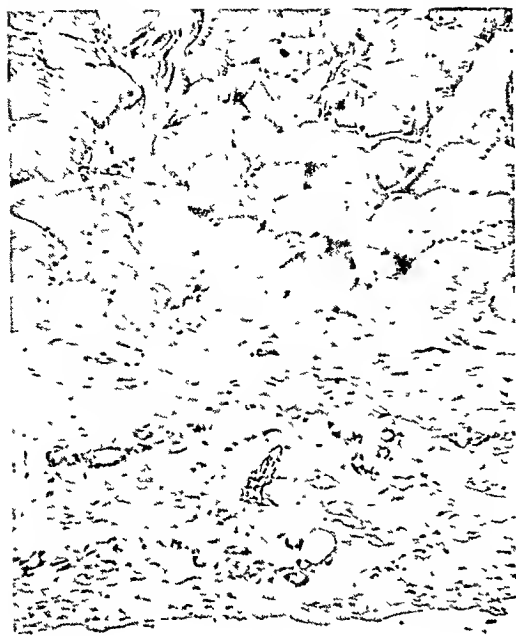


Fig. 1.—Section through the skin area adjacent to the gas space. The gas space is lined by a uniform organized connective tissue wall which shows acidophilic staining connective tissue with numerous capillaries and which, with Mallory stains, reacts positively for collagen.

From a thorough histological study, the surviving rats were found to exhibit the lesions commonly found in rats of this age in our colony. Low grade chronic pulmonary infection and low grade kidney infection were often seen. A number of parasites were observed in the liver and the urinary bladder. Only one tumor was found—a sarcoma of undetermined origin. One unusual lesion was described as a hamartoma of loose connective tissue with prominent capillaries and a few irregularly arranged epithelial cells forming duct-like structures.

A special search was made for any effects that might be attributed to a contamination with trace amounts of SF_6 . This compound is known to produce pulmonary edema, and questionable damage to the epithelium of large bronchi and to the trachea when the gas is inhaled (11). None of these changes could be found in any of the rats examined.

In summary, groups of 20 male and 20 female, young adult, albino rats were treated and observed over a period of six months. Each experimental rat was injected with a volume of SF_6 , either intraperitoneally or subcutaneously. Each control rat was injected with a similar volume of air. The injections were repeated for both groups simultaneously. Infections were controlled with injections of penicillin to obviate deaths following the treatment days. Mortalities were comparable for the control and experimental rats. The organ weights of rats surviving the six-months' period lay in normal ranges. A thorough histological study re-

vealed no evidence of any tissue lesion that was attributed to the treatment. SF_6 was neither toxic nor irritating under the conditions of this study.

DOG STUDIES

Two dogs were maintained with pneumoperitoneum refills for periods of six months or longer. A third dog that died after the second injection of SF_6 showed on autopsy evidence of what is taken to be an intercurrent illness, *viz.*, a focal bronchial pneumonia, a generalized pulmonary congestion, hemoperitoneum, and hemopericardium.

The two dogs that survived the six months' experimental period were treated on the schedule shown in Table III. Gas was introduced into the peritoneal cavity by a 100-cc. syringe and a semi-blunt needle equipped with a simple rubber bag containing the gas. Injections were always made at approximately the same site in the lower quarter of the abdomen. Before introducing the SF_6 , the residual gas within the peritoneum was removed. These two dogs were normal, young, well-nourished male beagles. The dogs had good appetites during the test period. During a period of several months, one dog lost weight, *viz.*, the body weight fell from 11.8 to 9.7 Kg.; some of this weight was regained before the end of the experimental period.

TABLE III.—TREATMENT PROGRAM FOR DOGS, BODY WEIGHTS

	Dates of SF_6 Refills	Body Wt (Kg)
Dog No. 2999 M	5-12-54	14.8
	6-9-54	15.2
	7-14-54	15.9
	7-17-54	Dead
Dog No. 2081 M	Initial	11.8
	3 mo.	10.9
	6 mo.	10.4
	9 mo.	9.7
	Termination	10.0
Dog No. 2823 M	Initial	6.3
	3 mo.	6.6
	6 mo.	6.1
	Termination	6.3

Blood counts were obtained for each dog toward the end of the experimental period. The hematological values recorded included red blood cell counts, hemoglobin values, white blood cell counts, differential counts, and observations of a number of characteristics of the red blood cells. All values were in the normal ranges.

Urine samples were collected from one of the dogs (No. 2823) and examined by the usual semiquantitative methods for sugar and protein—only normal trace amounts were present.

Organ Weights.—The dogs were sacrificed by the intravenous administration of a large dose of a soluble barbiturate. A number of organs were dissected out and weighed (Table IV). The organ weights were within the normal ranges.

Pathology.—When the dogs were sacrificed, gross autopsy examinations were made by Dr. R. D. Neubecker of the Department of Pathology. Sections of the following tissues were made and stained with the customary hematoxylin and eosin

TABLE IV.—ORGAN WEIGHTS OF DOGS

	Organ Wt. (fresh)		Organ Wt. Basis Body Wt. (Gm./kg.)	
	No. 2081	No. 2823	No. 2081	No. 2823
Heart	90	46	9.0	7.3
Lungs	R 53	38	5.3	6.0
	L 40	30	4.0	4.8
Spleen	43	28	4.3	4.4
Liver	314	234	31.4	37.1
Kidneys	R 41	24	4.1	3.8
	L 41	24	4.1	3.8
Brain	78	67	7.8	10.6

stains: heart, lung, spleen, pancreas, stomach, small and large intestine, liver, kidney, urinary bladder, lymph nodes, omentum, peritoneum, diaphragm, abdominal wall, adrenal, testes, thyroid, bone marrow, and brain.

Neither dog showed any gross or histological changes that were attributed to the SF₆ treatment. One dog (No. 2081) showed a mild pyelitis and had focal granulomata in the lung and lymph nodes. The pleural cavities were normal. No excess fluid was present in the peritoneal space. The peritoneal surfaces were thin and normal. Specifically there was no inflammation or scarring in the omentum, or the diaphragm, or in the tissues most closely exposed in the peritoneal cavity and in the pleural cavity. One dog had some cystitis, a mild pyelitis, and a vesicle calculus. Lung sections were normal with the exception of some scattered intra-alveolar hemorrhage.

Both of these dogs were normal, well-developed young dogs with no histological evidence of any effect of SF₆. As with the rats, a careful search revealed no indication that a material having the properties of S₂F₁₀ had been present in any detectable trace.

DISCUSSION

Based on the evidence of the lack of toxicity in the animal studies, two clinical trials were conducted (10): one series on three volunteer patients at the Iola Sanitarium in Rochester, N. Y.; the other on ten patients undergoing treatment for tuberculosis in the Veterans' Hospital, Batavia, N. Y.

In Table V, the records of treatment periods, volumes of gases, and frequencies of refills are presented. The patients were examined fluor-

scopically to observe the volume changes of the gas pockets and the gas pressures were measured directly before and after each refill. No untoward symptoms or physical signs were recorded during the course of SF₆ pneumoperitoneum. The longer periods between refills, *viz.*, five or six weeks with SF₆ vs. ten to fourteen days with air, and the lessened discomfort because needle injections were less frequent were greatly appreciated by the patients.

The patients in the Veterans' Hospital group received SF₆ injections over periods of three to nine months. Refill volumes of 800 to 1,000 cc. were required at intervals of five to seven weeks, in contrast to air refill volumes 100 to 200 cc. larger at intervals of ten to fourteen days. Records of temperature, pulse, hemoglobin, white blood cell count, bromsulphalein retention, blood urea nitrogen, and phenolsulfonphthalein tests were negative, *i. e.*, functions were unimpaired.

As a result of the preliminary trials, SF₆ is recommended (10) as "an ideal gas for use in therapeutic artificial pneumoperitoneum and should also be tried clinically in artificial pneumothorax."

SUMMARY

1. Groups of 20 male and 20 female rats, each, were treated (a) with SF₆ subcutaneously, (b) with SF₆ intraperitoneally, (c) with air subcutaneously, and (d) with air intraperitoneally, for a period of approximately six months. The intervals between injections varied from one to two weeks. Each group grew normally.

2. Deaths, presumably from infection, occurred during the early part of the experiment; the administration of penicillin simultaneously with the air or SF₆ injections controlled the mortalities. Mortality rates were no greater when SF₆ was administered than when air was administered.

3. Organ weights lay in the normal ranges.

4. In a thorough histological study, no lesions were found that were attributed to the treatment by SF₆. Specifically, the skin and membranes that had been in contact with SF₆ or with air showed no evidence of toxicity or irritation.

5. Two dogs were maintained with pneumoperitoneum of SF₆, one dog for ten months and the other dog for nearly seven months. Gas was administered at intervals varying from one week

TABLE V

	Air	SF ₆
Iola Case No. 1	March 1951–Sept. 1952 1,200 cc. every 10 days	Sept. 1952–April 1954 1,000 cc. every 6 weeks.
Iola Case No. 2	July 1951–Sept. 1952 1,000 cc. every 10 days April 1954– 1,000 cc. every 2 weeks	Sept. 1952–April 1954 800–900 cc. every 5 weeks.
Iola Case No. 3	April 1954 1,400 cc. every two weeks	Oct. 1953–April 1954 1,200 cc. every 5 weeks.

to three weeks. Body weights were maintained by one dog, the other dog lost some weight but regained most of it by the end of the period. Organ weights lay in the normal ranges. A thorough histological study revealed no tissue change that was attributed to the administration of SF₆.

REFERENCES

(1) Wehh, G., Gilbert, G., James, T., and Havens, L., *Arch Internal Med*, 14, 883(1914)

- (2) Grass, H., and Meiners, H. H., *Beitr Klin Tuberk.*, 51, 134(1922)
- (3) Schedtler, O., *Klin Wochschr.*, 17, 1153(1938)
- (4) Risen, H., *U. S. Naval Med Bull.*, 40, 853(1942)
- (5) Bénard, H., and Camus, L., *Compt rend. soc biol* 104, 1276(1930)
- (6) Teschendorf, W., *Arch expil Pathol Pharmacol* 104, 352(1924)
- (7) Coryllos, P. N., and Birnbaum, G. L., *Arch Surg*, 21, 1214(1930)
- (8) Canfield, R., and H. Rahn, (unpublished data)
- (9) Tenney, M. S., Carpenter, F. G., and Rahn, H. *J Appl Physiol*, 6, 201(1953)
- (10) Swalbach, W. G., Hodge, H. C., Schwartz, S. I., and Rahn, H., in press
- (11) Saunders, J. P., Shoshkes, M., DeCarlo, M. R., and Brown, E. C., *A. M. A. Arch Ind Hyg and Occupational Med*, 8, 436(1953)

A Study of the *In Vitro* Reaction of Catalase and Hemin with Organic Mercury Compounds*

By SISTER M. ANGELICE SEIBERT

The relative inhibitory effect of a series of organic mercury compounds (phenylmercuric nitrate, phenylmercuric hydroxide, *p*-chloromercuribenzoic acid, Neohydrin, and Mereuhydrin) on the catalytic activity of catalase *in vitro* was studied. The inhibition is relatively less with the diuretics. Increasing the concentration of all of the mercury compounds gives greater inhibition. Spectrophotometric studies of catalase protein, Armour serum albumin and crystalline hemin were made. Interaction of the protein —SH with the organic mercury compounds does not explain the inhibition under the conditions of this study. The catalase activity of hemin is inhibited by the mercury compounds in a system completely devoid of —SH groups. The hemin itself is rapidly destroyed by the hydrogen peroxide and this reaction is also depressed by the organic mercury compounds. There is no spectroscopic evidence for a hemin-mercury or hydrogen peroxide-mercury compound. Spectroscopic and preliminary polarographic data indicate the necessity of postulating a ternary complex, hemin-hydrogen peroxide-organic mercury, which is relatively stable.

AT PRESENT there is a variety and unfortunate confusion of concepts relating to the mode of action *in vitro* and *in vivo* of several types of mercury compounds falling under the classification of organic mercury compounds

p-Chloromercuribenzoic acid (PCMB) has been considered a selective reagent for —SH binding and its reversible inhibition of enzyme activity has led to the expression "sulfhydryl-dependent enzymes" (1). However, the simple inhibition of enzyme activity by PCMB does not demonstrate conclusively that —SH groups are essential for the activity of a given enzyme (2, 3).

Phenylmercuric nitrate (PMN) and phenylmercuric hydroxide (PMOH) have been shown to inhibit *in vitro* in a non-reversible manner

enzymes supposedly not requiring —SH groups for activity (4). The *in vitro* studies of Goldstein and Doherty (5) with cholinesterase indicate that the problem of the influence of mercury compounds on enzyme activity is not adequately explained in some instances in terms of —SH binding. The demonstration of a direct interaction of organic mercury compounds with thyroxine and related compounds under mild conditions by Frieden and Naile (6) is also of interest to this problem.

Organic mercury compounds of the diuretic group inhibit both —SH and non—SH dependent enzymes (7). Ruskin and Ruskin (8) have suggested that the action of Mereuhydrin may be at two different sites of the terminal oxidative chain. Riggs and Wolbach (9) investigating the inhibitory effects of organic mercury compounds on the autocatalytic binding of hemoglobin with oxygen conclude that the mercury compounds inhibit the heme-heme interaction by blocking or modi-

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fying structural reorganization which accompanies the oxygenation

Earlier work (4, 10, 11) has shown that catalase is inhibited by PCMB, PMN and PMOH. This inhibition is relatively mild as compared with that observed with cytochrome oxidase and succinic dehydrogenase. Since this inhibition of catalase was apparently not a simple binding of essential —SH groups, it was of interest to investigate further the mode of action of these mercury compounds and to compare the effects of these compounds with those of several organic mercury diuretics.

EXPERIMENTAL

All spectrophotometric measurements were made with a Beckman Model DU spectrophotometer employing a water cooled lamp house and cell compartment.

Polarographic measurements were made with a Sargent Model XII Camera Recording Polarograph. An H cell with a saturated calomel electrode and a KCl agar bridge was used as the anode. Oxygen was removed from the test solutions by passage of oxygen-free nitrogen. All measurements were carried out at $25 \pm 0.1^\circ$. The capillary had the following characteristics: $t = 3.3$ sec at a pressure of 48 cm of mercury on an open circuit in the test solution. The weight of the mercury dropping per second, m , was 2.04 mg. All solutions were buffered to a pH of 7.4 with 0.1 *M* phosphate buffer and were examined over a potential range of 0.0 to -1.8 v.

Armour No. 30 pork liver catalase¹ was used for all the enzyme experiments. Crystalline hemin was purchased from Nutritional Biochemicals.

The stock solution of catalase was prepared by dissolving a weighed amount of the powdered enzyme in 0.1 *M* phosphate buffer, pH 7.4, and diluting to the required concentration for use. The final concentration of the enzyme was determined spectrophotometrically at 406 $m\mu$ as described in a previous paper (4).

The hemin crystals were dissolved in a few drops of 6 *N* NaOH and several ml. of glass distilled water. The solution was then made up to volume with buffer. The concentration was calculated from an accurately weighed sample of crystals using a molecular weight of 675.55. The stock solution was 1.4×10^{-5} *M*.

Basic phenylmercuric nitrate and phenylmercuric hydroxide were purchased from Hamilton Laboratories, Inc., PCMB from the Schreffer Laboratories, Inc. Mercurhydrin and Neohydrin were obtained from Lakeside Laboratories, Inc., Wisconsin.¹

Catalase and hemin activity was determined by following the breakdown of hydrogen peroxide at 240 $m\mu$ according to the tracking method of Beers and Sizor (12) with some modifications.

In all the experiments the catalase or hemin and the inhibitor were premixed and allowed to stand for one hour before adding the substrate. The catalase solutions were kept at 10° at all times,

the hemin solutions at 25° .

An approximately 4.5×10^{-3} *M* H_2O_2 solution was prepared by diluting 12 ml. of Mallinckrodt, A.R., 3% H_2O_2 with 25 ml. of 0.1 *M* phosphate buffer pH 7.4, for the catalase experiments. For the hemin experiments a 8.8×10^{-2} *M* solution was used.

In the catalase experiments 2 ml. of the enzyme solution were placed in both the blank and experimental cell. One ml. of buffer was added to the blank. At zero time, 1.0 ml. of the H_2O_2 was added to the enzyme solution and the absorbance at 240 $m\mu$ read every ten seconds for a ninety second period.

In the hemin experiments, 3.0 ml. of the hemin solution was added to the experimental cell. The blank contained 3.0 ml. of either buffer or of the buffer and mercury compound. At zero time, 0.5 ml. of H_2O_2 was added to the experimental cell and the change in absorbance was followed with time. Final concentration of the hemin in the spectrophotometer cell was 2.7×10^{-5} *M*.

RESULTS AND DISCUSSION

Catalase studies. Table I shows the comparative effect of five organic mercury compounds on the activity of Armour catalase No. 30. The per cent depression is calculated from the rates of change of the absorbance during a one minute interval (from twenty to eighty seconds) of a control catalase solution and one containing the stated molar concentration of mercury compound. Concentration of the mercury compounds are final molar concentration in the reaction mixture.

TABLE I. DEPRESSION OF CATALASE ACTIVITY BY ORGANIC MERCURY COMPOUNDS

Hg Compd (1.1×10^{-4} <i>M</i>)	% Depression
Phenylmercuric nitrate	23
<i>p</i> -Chloromercuribenzoic acid	16 ^a
Phenylmercuric hydroxide	8
Neohydrin	5
Mercurhydrin	0

^a This depression was obtained with a concentration of 1.1×10^{-4} *M*. Higher concentrations were too absorbing to permit spectrophotometric measurements.

The concentration of catalase in each of the reaction mixtures was 1.12×10^{-5} *M*. Increasing the concentration of the mercury compounds gave increased inhibition. Mercurhydrin could not be used in concentrations greater than 5×10^{-4} *M* because of high absorbance. The effect of mercuric amounts of Neohydrin is shown in Fig. 1.

Activity experiments measuring the effect of the mercury compounds on the ability of hemin to break down H_2O_2 showed significant inhibition of this property of hemin. Table II summarizes these data. The final molar concentration of the mercury compounds was 1.4×10^{-4} *M*, hemin, 2.7×10^{-5} *M*, H_2O_2 , 1.25×10^{-2} *M*. The temperature was 25° .

The effect of varying the concentration of Neohydrin and PMN is shown in Fig. 2.

A study of possible mercaptide formation between the mercury atom of the organic mercury compounds

¹ These materials were generously supplied without charge.

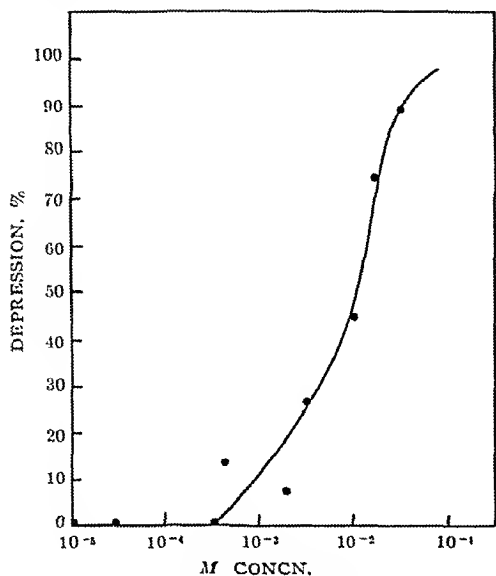


Fig. 1.—Effect of Neohydrin on the activity of Armour Catalase No. 30.

TABLE II.—DEPRESSION OF THE CATALYTIC ACTIVITY OF HEMIN BY ORGANIC Hg COMPOUNDS

Hg Comps.	Depression, %
Phenylmercuric nitrate	75
Neohydrin	50
Phenylmercuric hydroxide	37
Mercurhydrin	14

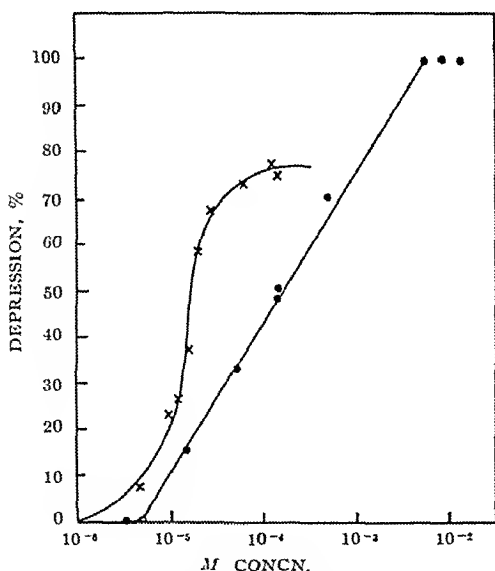


Fig. 2.—Effect of organic mercury compounds on the catalytic activity of crystalline hemin. ● Neohydrin; X Phenylmercuric nitrate.

and the —SH groups of catalase protein and Armour bovine serum albumin¹ indicated that under the conditions of these experiments very little mercaptide formation occurs as judged by increase of absorbance of the solutions at 250 $m\mu$. Measurements were made according to the procedure used by Boyer (14). In any case the concentration of mercury compound employed in the catalase activity experiments is in considerable excess of that required for —SH binding. For these studies Armour catalase No. 30 was used without further purification. This preparation contains only 24% catalase protein. Therefore, in order to prepare a solution of catalase protein an amount 24% greater than the calculated amount had to be dissolved. This resulted in a protein solution having a very high nonspecific protein absorbance. The very small increase in absorbance obtained in mixtures of this solution and the mercury compounds could be attributed almost completely to a non-specific reaction of the mercury compounds with the non-catalase protein. This interpretation is substantiated by earlier work (4) which showed that a very pure crystalline catalase preparation when mixed with PMN or PMOH showed no spectral shifts nor absorbance increase over the entire spectral range from 230 $m\mu$ to 700 $m\mu$. Part of the action of the mercury compounds must then involve the hematin portion of the catalase molecule.

It was reported in an earlier publication that the hemin spectrum is not affected by relatively high concentrations of PMN and PMOH and PCMB (4). This is in agreement with the observations of Riggs and Wolbach (9) that neither mercuric chloride, Mersalyl, PCMB nor methyl mercury hydroxide alter the spectrum of free ferriheme. Benesch and Benesch (13) also report that PMOH and PCMB have no effect on the absorption spectrum of hemoglobin.

Measurements of the absorption spectra of mixtures of the mercury diuretics, Neohydrin and Mercurhydrin, with hemin between 230 $m\mu$ and 650 $m\mu$ showed no alteration of the hemin or mercury compound spectra. Likewise no spectral changes were demonstrated in mixtures of the mercury compounds with hydrogen peroxide. Mixtures of hemin and hydrogen peroxide and of hemin, hydrogen peroxide and PMN in concentrations used in the activity experiments showed no differences in absorption spectra with the exception of a drop in absorbance due to the decomposition of the hydrogen peroxide and of the hemin. The spectra were run within five minutes after adding the hydrogen peroxide. Haurowitz, *et al.* (15), report a short-lived addition product of ferri-heme with hydrogen peroxide in aqueous media. This product is stabilized in pyridine and gives a characteristic absorption spectrum.

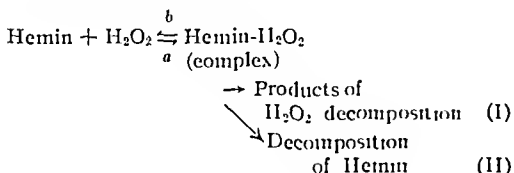
Under the conditions of our experiments, hemin is rapidly destroyed by the hydrogen peroxide during the reaction as shown by spectral evidence. The rate of destruction of hemin was measured by the same method as was used in following the breakdown of hydrogen peroxide except that the absorbance was read at the 390 $m\mu$ and 580 $m\mu$ maxima of hemin. The change in absorbance during the thirty minute period from five to thirty-five minutes is shown in Table III. The decomposition of hemin

is considerably faster at 25° than at 10° and the PMN inhibition of the decomposition reaction is relatively greater at the higher temperature

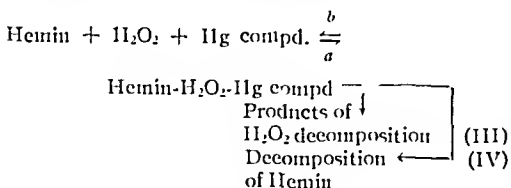
TABLE III EFFECT OF PMN ON THE DESTRUCTION OF HEMIN BY HYDROGEN PEROXIDE

	Δ In Absorbance 10°	at 390 mμ 25°	580 mμ 25°
Hemin	0.308	0.638	0.075
Hemin-PMN	0.233	0.380	0.040

The reaction of hemin and hydrogen peroxide would appear to be as follows



Normally in the case of catalase reaction (I) is faster than reaction (II). Our data with hemin show that reaction (II) is the faster under the conditions stated. The organic mercuric compounds inhibit both reactions (I) and (II). It does not seem necessary nor justifiable to postulate two different modes of action for the mercury compounds. In both instances they apparently work by preventing the breakdown of the hemin-H₂O₂ complex. Since we have no evidence for a hemin-mercury or a hydrogen peroxide-mercury complex, the mercury compounds apparently do not inhibit reaction (a). Inhibition of (b) would result in the accumulation of the hemin-hydrogen peroxide complex making it observable spectroscopically. For this we have no evidence. The data, then, suggest a ternary compound which does not decompose as rapidly as reaction (I) or (II) thus inhibiting both the catalytic property of the free hemin molecule and the decomposition of hemin by hydrogen peroxide.



The rate of hydrogen peroxide breakdown by catalase is constant both in the control and mercury treated solutions. The rate of the hemin reactions (I) to (IV) decreases with time due to the simultaneous decomposition of the hydrogen peroxide and the hemin.

This hypothesis of a ternary complex is supported

by evidence from preliminary polarographic analyses. Polarograms of mixtures of the mercury compounds ($6.5 \times 10^{-4} M$) and hemin ($2.2 \times 10^{-3} M$) showed no shift in half wave potentials. The $E_{1/2}$ for hydrogen peroxide ($1.25 \times 10^{-2} M$) was -0.849. When hemin was added to the half cell with the H₂O₂, the $E_{1/2}$ shifted to -0.458. The hemin catalyzes the reduction of the hydrogen peroxide thereby lowering the energy necessary for the polarographic reduction of the latter. The addition of PMOH to the cell mixture shifts the $E_{1/2}$ negatively to -0.714. This negative shift suggests the formation of a complex which requires a greater amount of energy for reduction than the hemin-hydrogen peroxide complex.

SUMMARY

Two mercurial diuretics, Neohydrin and Mercurhydrin, inhibit the catalytic activity of catalase *in vitro*. This inhibition is compared to that given by the same concentrations of other organic mercury compounds.

The organic mercury compounds also inhibit the catalase activity of purified hemin in a system completely devoid of -SH groups. The destruction of hemin by hydrogen peroxide is also inhibited by these compounds.

Spectroscopic and polarographic data indicate the necessity of postulating a hemin-hydrogen peroxide-organic mercury complex which is relatively stable.

The inhibition of the enzymatic activity of catalase is suggested by analogy to involve the formation of a catalase porphyrin-hydrogen peroxide-organic mercury complex.

REFERENCES

- (1) Barron, E. S. G., *Advances in Enzymol.*, **11**, 201 (1951).
- (2) Onrust, H., Van der Linden, A. C., and Jansen, B. C. P., *Enzymologia*, **16**, 19 (1951).
- (3) Swenson, A. D., and Boyer, P. D., *J. Am. Chem. Soc.*, **79**, 2171 (1957).
- (4) Sollier, Sr. M. R., Seibert, Sr. M. A., Kreke, C. W., and Cook, E. S., *J. Biol. Chem.*, **198**, 281 (1952).
- (5) Goldstein, A., and Doherty, M. E., *Arch. Biochem. Biophys.*, **33**, 35 (1951).
- (6) Frieden, E., and Nade, B., *ibid.*, **45**, 338 (1952).
- (7) Ruskin, B., Nowinski, W. W., and Ruskin, A., *Texas Repts. Biol. Med.*, **8**, 381 (1950).
- (8) Ruskin, A., and Ruskin, B., *J. Pharm. Exptl. Therap.*, **107**, 325 (1953).
- (9) Riggs, A. F., and Wolbach, R. A., *J. Gen. Physiol.*, **29**, 583 (1956).
- (10) Cook, E. S., Kreke, C. W., McDevitt, Sr. M. L., and Bartlett, Sr. M. D., *J. Biol. Chem.*, **162**, 43 (1946).
- (11) Kreke, C. W., Schaefer, Sr. M. A., Seibert, Sr. M. A., and Cook, E. S., *ibid.*, **185**, 169 (1950).
- (12) Beers, R. F., Jr., and Sizer, I. W., *ibid.*, **195**, 133 (1952).
- (13) Benesch, R. E., and Benesch, R., *Arch. Biochem. Biophys.*, **48**, 38 (1951).
- (14) Boyer, P. D., *J. Am. Chem. Soc.*, **76**, 133 (1954).
- (15) Harrowitz, F., Brdicka, R., and Kraus, F., *Enzymologia*, **2**, 9 (1937).

The Spectrophotometric Determination of Sterols in Wool Fat*

By BLAKE F. PUTNEY and ROBERT CALVO

Irregularities encountered in the application of the Liebermann-Burchard reaction to the determination of cholesterol, ischolesterol and their esters in wool fat unsaponifiables and wool fat have been investigated. Times and temperatures of color formation have been found critical in the determination of absorptivities and their analytical application. A satisfactory analytical procedure has been developed.

THE WIDE USE of wool fat, its derivatives and fractions, in cosmetics and medicinals has necessitated a reliable method for determining the wool fat content of these preparations. The difficulty is that wool fat is a complex mixture of many constituents, which vary in their relative proportions depending on the source of the wool and also on the process of extraction and purification. The monograph of wool fat U. S. P. is scarcely more than a simple physical description of the product and gives little indication of its chemical composition.

The commercial availability of several wool fat fractions suggested to the authors that various studies be carried out on these fractions to gain some insight into their chemical composition. The spectrophotometric determination of cholesterol and ischolesterol appeared to be a good starting point for a chemical investigation of these products.

The chemistry of wool fat has been reviewed by Lederer and Velluz (1) and Truter (2). The name, ischolesterol, as originally proposed for the steroid component found in wool fat unsaponifiable (in addition to cholesterol) has now been shown to be a mixture of four distinct compounds; namely, lanosterol (lanostadienol), dihydrolanosterol (lanostenol), agnosterol, and dihydroagnosterol. The mixture has also been referred to quite commonly as "the triterpene alcohols of wool wax." In this report, the name ischolesterol, refers to the mixture of the triterpene alcohols described above. Some confusion also exists in the use of the name, lanosterol, in that the wool fat component to which it originally referred has now been shown to be an equimolecular mixture of lanosterol and dihydrolanosterol. The term, lanosterol, is used here to refer to the single alcohol component, also known as lanostadienol which is identical with kryptosterol.

The Liebermann-Burchard reaction for quantitative determination of cholesterol was first proposed by Grigaut in 1910, and has since been used extensively with many modifications (3) to determine the cholesterol content of the blood and other body fluids. Other spectrophotometric methods for determinations of cholesterol in blood have been reported by Pearson (4) and Zlatkis (5); no report was found of the use of the latter methods in determination of cholesterol or ischolesterol in wool fat. A gravimetric method for cholesterol in blood has also been widely used, but its use in wool fat analysis has been seriously questioned by Knol (6).

The different colors produced by cholesterol and ischolesterol when treated with L.-B. reagent were first observed by Schulze in 1890, and studied extensively by Lederer and Tchen (7, 8). The latter observed that, essentially, the same color reaction was given by the other sterols of the ischolesterol fraction as given by lanosterol, and suggested the use of lanosterol as a standard for the determination of the ischolesterol content of wool fat.

The application of the L.-B. reagent to cholesterol esters has shown that the color is produced more rapidly and that the absorbance is greater by approximately 20 per cent for equivalent cholesterol content than in the case of free cholesterol (9). Similarly, Lederer and Tchen (7) reported a difference of about 5 per cent in the color produced by lanosterol and lanosteryl acetate.

A recent publication by Luddy, *et al.* (10), reported extensive work on the determination of cholesterol and ischolesterol (triterpene alcohols) in wool fat. The chief modifications in this procedure involved the determination of ischolesterol at 550 $m\mu$, instead of the wavelength of the primary maximum at 450 $m\mu$ (Fig. 1); use of a spectrophotometer; and development of the color complexes at room temperature. This procedure was questioned because the temperature was not controlled and standard solutions

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Presented to the Scientific Section, A.P.A., New York meeting, May 2, 1957.

of cholesterol and lanosterol were not run under the same conditions of time and temperature as the unknown solutions. Ducwell (11) has also reported use of the L-B reaction for determination of cholesterol and ischolesterol in wool fat.

EXPERIMENTAL

Equipment.—A Bausch and Lomb Spectronic-20 Spectrophotometer, equipped with a Thomas Roto-Cell Carrier Assembly¹ with a partitioned Corex cell having a light path of 1 cm., was used. The carrier was equipped with a liquid cooling chamber through which water was passed in order to maintain the desired temperature.

The samples were prepared in glass stoppered 25-cc. mixing cylinders of low actinic glass.

Reagents.—The cholesterol (Fisher Scientific Co.) was recrystallized from alcohol-water and dried at 80° for three hours, $m.p. 143.5-144.0^\circ$, $[\alpha]_{25}^D -38.8^\circ$ ($CHCl_3$).

Cholesteryl acetate (Brothers Chemical Co.) was recrystallized from acetone and dried at 80° for three hours, $m.p. 113.5-114.0^\circ$.

Initially, the lanosterol used was obtained by isolation from wool fat by the method of Dörce and Garratt (12), $m.p. 138-138.5^\circ$. Later, a sample of "pure lanosterol" (Organon Laboratories Ltd., London, England)² $m.p. 137.5-138.0^\circ$, $[\alpha]_{25}^D +56.5^\circ$, was used.

Lanosteryl acetate was prepared by refluxing lanosterol with an excess of acetic anhydride, crystallized from alcohol or acetone and dried at 80° for three hours, $m.p. 123-125^\circ$. The acetate prepared from the commercial lanosterol, after drying at 80° for three hours, had a $m.p.$ of 126-128°.

Special peroxide free 1,4-dioxane (Fisher Scientific Co., cat. no. D-111) was used.

Reagent grade chloroform, sulfuric acid, acetic anhydride, acetic acid, and wool fat, U.S.P., were used.

The Liebermann-Burchard reagent was prepared by the method of Luddy (10). 1 volume of cold (0°) concentrated sulfuric acid was added to 4 volumes of acetic anhydride immersed in an ice-salt bath. The acid was added slowly with shaking to prevent discoloration of the reagent at this point. The acid-anhydride mixture was then diluted with 2.5 volumes each of glacial acetic acid and 1,4-dioxane and stored at 0°. The reagent is relatively unstable and should only be used on the day of its preparation. It should be rejected if a brown color develops during use.

Stock solutions.—Stock solutions of the sterols and their acetates were prepared in reagent grade chloroform. The solutions had concentrations in the range of 6 to 12 mg. of sterol in 100 cc. of solution. Wool fat solutions were prepared to contain 25 to 35 mg. of wool fat in 100 cc. of solution. The solutions of wool fat unsaponifiable were made to

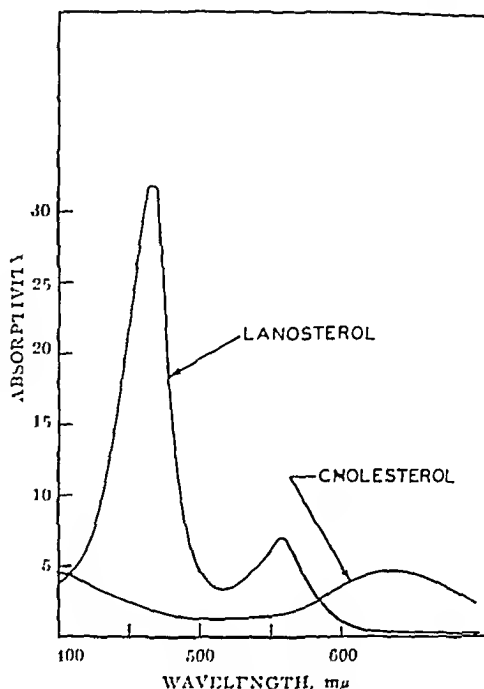


Fig. 1—Plot showing the wavelength of maximum color absorption of the Liebermann-Burchard color complexes of cholesterol and lanosterol (10).

contain 12 to 16 mg. of unsaponifiable in 100 cc. of solution.

Procedure.—Ten cubic centimeters of the solution to be analyzed was pipetted into a 25 cc. mixing cylinder and 5 cc. of cold L-B reagent was added. A blank consisting of 10 cc. of chloroform and 5 cc. of L-B reagent was prepared simultaneously. Immediately after adding the reagent, a stop watch was started, the cylinder inverted several times and placed in a constant temperature bath, maintained at 30° and protected from light. The length of time for a sample in the bath varied, as indicated in Table I, in order to allow the color to reach its maximum intensity after the solution was placed in the spectrophotometer. Water from the bath, at 30°, was passed through the cooling chamber of the carrier assembly.

Absorbances were taken at two-minute intervals, reading first at 550 $m\mu$ and then at 630 $m\mu$. During the assay, the cell was turned so that light passed through the solution *only while readings were being taken*.

Calculations.—In determining the absorptivities, the absorbances of cholesterol, lanosterol, and their respective acetates were taken at 630 $m\mu$ (the wavelength at which maximum light absorption occurs for cholesterol and its esters) and at 550 $m\mu$ (the wavelength at which a secondary maximum of absorption occurs for lanosterol and its esters) Fig. 1. This was done in order to take into account the colored ischolesterol derivatives having appreciable absorption at the wavelength

¹ A. H. Thomas Co., Phila., Pa.

² All $m.p.$'s are corrected.

³ The authors wish to express their appreciation to Mr. E. A. Newcomb of the Malmstrom Chemical Corp., Newark, N. J., for obtaining this sample.

at which the cholesterol color complex has its maximum and vice versa. From these data the absorptivity was calculated as follows:

$$\text{Absorptivity } (a_L) = \text{Absorbance/Concn. (Gm./L.)} \\ \times \text{length of light path (cm.)}$$

These absorptivities (Table II) were then used in the calculation of cholesterol-cholesterol mixtures (Table III) and cholesterol-cholesterol mixtures (Table IV). The following equations were employed to determine the amounts of the respective sterols in the solutions: Cholesterol (Gm./L.) = $(A_{630}a_L - A_{550}a_L')/(a_La_C - a_L'a_C')$; Lanosterol (Gm./L.) = $(A_{550}a_C - A_{630}a_C')/(a_La_C - a_L'a_C')$ in which A_{630} and A_{550} are the absorbances of the solution at the respective wavelengths; a_C and a_C' are the absorptivities of cholesterol (or chole-

sterol acetate) at 630 and 550 $m\mu$, respectively; a_L and a_L' are the absorptivities of lanosterol (or lanosterol acetate) at 550 and 630 $m\mu$ respectively.

In order to determine the significance of the rate of color development on the results obtained in the following analyses, the calculations were carried out in two ways

Method I. —The absorbances employed in the calculations were those observed at the time of maximum color development for the sterols being determined. For example, on calculation of a mixture of cholesterol acetate and lanosterol acetate, the absorbances were those observed at 18 minutes at 630 $m\mu$ and 30 minutes at 550 $m\mu$. The absorptivities employed were those previously found at the corresponding times (Table II), a_C and a_L' at 18 minutes and a_L and a_C' at 30 minutes. In the analysis of cholesterol-cholesterol mixtures, absorbances

TABLE I.—TIME ALLOWED FOR COLOR DEVELOPMENT AND READING OF ABSORBANCE OF SOLUTIONS

Sample	Time in bath Min	Reading Times, Min		Maximum Color Time, Min	Maximum Color Wavelength $m\mu$
		Init	Final		
Cholesterol	18	20	32	24	630
Cholesteryl Acetate	10	14	32	18	630
Lanosterol	20	24	32	28	550
Lanosteryl Acetate	15	18	34	30	550
Wool Fat or Acetate mixtures	10	14	32	18	630
				30	550
Wool fat unsaponifiables or free sterol mixtures	18	20	32	24	630
				28	550

TABLE II.—ABSORPTIVITIES OF LIEBERMANN-BURCHARD COLOR COMPLEXES OF STEROL COMPONENTS OF WOOL FAT

Time, (Min)	Cholesterol		Chol Acetate		Lanosterol		Lano Acetate	
	630 $m\mu$	550 $m\mu$	630 $m\mu$	550 $m\mu$	630 $m\mu$	550 $m\mu$	630 $m\mu$	550 $m\mu$
14	5.35	1.77				
16	5.44	1.76			0.28	4.00
18	4.75	1.59	5.43	1.71			0.30	4.51
20	4.84	1.60	5.34	1.65			0.32	5.05
22	4.88	1.60	5.26	1.60			0.32	5.41
24	4.92	1.59	5.13	1.60	0.47	5.19	0.32	5.52
26	4.86	1.58	5.02	1.54	0.49	5.33	0.36	5.72
28	4.79	1.54	4.88	1.56	0.51	5.44	0.34	5.89
30	4.72	1.53	4.76	1.52	0.53	5.44	0.34	5.95
32	4.63	..	4.64		0.54	5.37	0.34	5.90
34		0.56	5.25	0.34	5.89

TABLE III.—RESULTS OF ANALYSIS OF CHOLESTEROL-LANOSTEROL MIXTURES AND OF WOOL FAT UNSAPONIFIABLES AND COMPARISON OF RESULTS OBTAINED BY TWO METHODS OF CALCULATION

Sample	Cholesterol			Lanosterol		
	Taken ^a	Found ^b , %	Method II ^c	Taken ^a	Found ^b , %	Method II ^c
147-A ^d	0.0243	100.3	100.3	0.0209	102.4	102.7
147-B ^d	0.0318	103.1	104.0	0.0390	100.6	100.4
147-C ^d	0.0492	102.4	102.6	0.0309	103.1	103.3
147-D ^d	0.0299	101.7	97.7	0.0390	100.3	103.0
Average		101.9	101.0	..	101.6	102.4
152-A ^e	0.1108	41.7	40.9	..	28.2	28.8
152-B ^e	0.1089	41.5	41.5	..	28.1	28.7
Average		41.6	41.2	..	28.2	28.8

^a The concentration of sterol (or wool fat unsaponifiable) in the color solution expressed as Gm./L.

^b Percentage of known amount of component, found on analysis of mixtures of sterols. For wool fat unsaponifiables, percentage of cholesterol or isocholesterol (calculated as lanosterol) in wool fat unsaponifiable.

^c See text. ^d Known mixtures of sterols. ^e Wool fat unsaponifiable.

were those observed at 24 minutes at 630 $m\mu$ and 28 minutes at 550 $m\mu$, absorptivities were also at 24 and 28 minutes, respectively

Method II.—In order to eliminate the long time for taking readings necessitated in Method I, calculations were made using the average time between the respective times of maximum color development. In the case of the acetate mixtures described above, the absorbances and the absorptivities were all taken at 24 minutes. In analysis of cholesterol-lanosterol mixtures, the average time of 26 minutes was used.

Calculation by Method I was found to give satisfactory results in all cases (Table III and IV). Method II gave satisfactory results for cholesterol, lanosterol and cholesteryl acetate, but for lanosteryl acetate, the results were much less precise than the results obtained by Method I. The two methods of calculation gave fairly consistent results when whole wool fat was analyzed for combined cholesterol and ischolesterol (as cholesteryl acetate and lanosteryl acetate).

DISCUSSION

In the determination of maximum color development under controlled temperature, it was found that there was a wide variation in the time necessary for maximum color development of cholesterol, lanosterol and their acetate esters (Fig. 2). Cholesteryl acetate develops its maximum in approximately 18 minutes, while free cholesterol develops its maximum color in about 24 minutes. As previously found by other investigators (9), the absorbance of the cholesteryl acetate color complex was about 20% greater than the absorbance produced by an equivalent amount of cholesterol. On the other hand, lanosteryl acetate was found to require approximately 30 minutes for maximum color development, while free lanosterol required only 28 minutes. As with cholesterol, the absorbance of the lanosteryl acetate color complex was found to be about 20% greater than the absorbance of an equivalent amount of lanosterol. Lederer and Tehen (7) had reported a difference of only 5% for lanosterol and lanosteryl acetate. This variation in time and extent of color development was found to be significant in the determination of the amounts of esterified cholesterol and lanosterol in mixtures of their acetates. The absorbances taken at an average time (24 minutes) appeared to be less accurate,

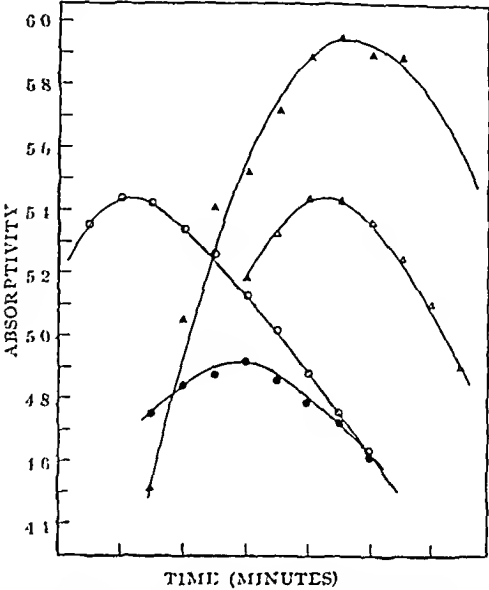


Fig. 2.—Plot showing the relationship of time to absorptivities of cholesterol, lanosterol, cholesteryl acetate and lanosteryl acetate. ● Cholesterol, 630 $m\mu$; ○ Cholesteryl acetate, 630 $m\mu$; ▲ Lanosterol, 550 $m\mu$; △ Lanosteryl acetate, 550 $m\mu$.

for lanosteryl acetate in particular, than the absorbances taken at 18 minutes for cholesteryl esters (wavelength 630 $m\mu$) and at 30 minutes for lanosteryl acetate (wavelength 550 $m\mu$) (Table IV). The difference of time for color development of unesterified cholesterol and lanosterol is much less, 4 minutes, and therefore, an average time (26 minutes) may be used without a great loss of precision (Table III, Fig. 3).

In order to determine the reliability of the analytical method on unknown samples of wool fat, these samples were analyzed, and the content of cholesterol and ischolesterol esters, calculated as cholesteryl and lanosteryl acetates was determined. After adding known increments of cholesteryl and lanosteryl acetates, the solutions were again analyzed. The concentrations of the new solutions were estimated on the basis of the sum of the pure ester (cholesteryl or lanosteryl acetate) added and the

TABLE IV — RESULTS OBTAINED ON ANALYSIS OF CHOLESTERYL ACETATE-LANOSTERYL ACETATE MIXTURES AND OF WOOL FAT AND COMPARISON OF RESULTS OBTAINED BY TWO METHODS OF CALCULATION

Sample	Cholesteryl Acetate Taken ^a	Found ^b , %		Lanosteryl Acetate Taken ^a	Found ^b , %	
		Method I ^c	Method II ^c		Method I ^c	Method II ^c
148-A ^d	0.0414	98.1	98.5	0.0274	102.5	107.1
148-B ^d	0.0505	99.1	100.6	0.0302	104.6	106.4
148-C ^d	0.0252	101.3	101.3	0.0549	102.4	109.7
Average		99.5	100.1	..	103.2	107.8
150-A ^e	0.2367	23.1	23.0	..	16.8	16.8
150-B ^e	0.1720	23.0	23.3	..	16.1	17.6
Average		23.1	23.1	..	16.4	17.2

^a The concentration of sterol (or wool fat) in the color solution expressed as Gm./L.

^b Percentage of known amount of component, found on analysis of acetate mixtures of cholesterol and ischolesterol esters in wool fat, calculated as cholesteryl or lanosteryl acetates.

^c See text.

^d Known mixtures of acetate esters.

^e Wool fat.

For wool fat, percentage of cholesterol and ischolesterol esters.

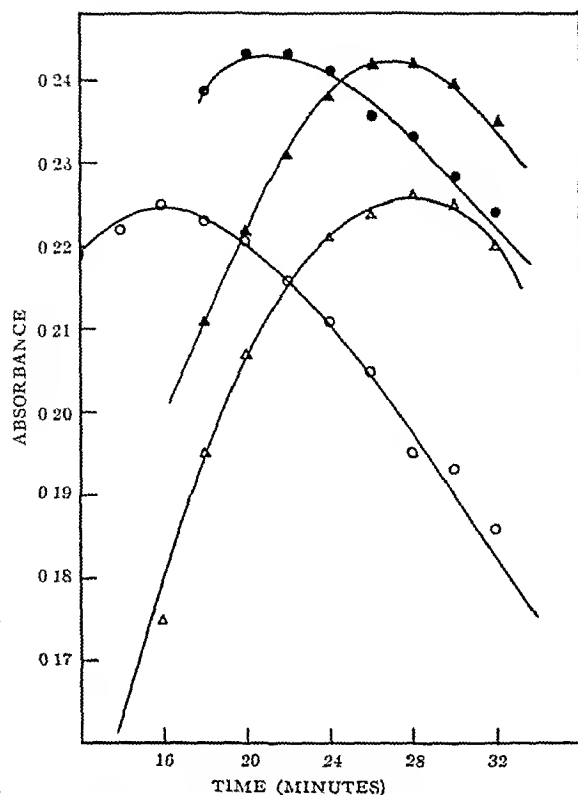


Fig 3—Plot showing the relation of time to the absorbances of sample runs of wool fat and wool fat unsaponifiable at 550 and 630 $m\mu$. \circ Wool fat, 630 $m\mu$, Δ Wool fat, 550 $m\mu$; \bullet Wool fat, unsap, 630 $m\mu$, \blacktriangle Wool fat unsap, 550 $m\mu$

TABLE V.—RESULTS OF ANALYSIS OF WOOL FAT PLUS ADDED AMOUNTS OF CHOLESTERYL AND LANOSTERYL ACETATES

Sample	Cholesterol Esters		Isocholesterol Esters	
	Taken ^a	Found ^b , %	Taken ^a	Found ^b , %
150-A-2	0 0647	100 9	0 0507	101 4
150-A-3	0 0748	102 0	0 0617	104 5
150-A-4	0 0697	103 7	0 0563	101 6
150-A-5	0 0596	102 4	0 0452	97 3
150-B-2	0 0446	99 8	0 0322	103 9
150-B-3	0 0496	100 8	0 0387	100 8
150-B-4	0 0547	102 0	0 0442	104 3
150-B-5	0 0597	100 8	0 0496	102 4
Average		101 5		102 0

^a The estimated concentration of solution, on basis of amount of cholesterol and isocholesterol esters (calculated as cholesteryl and lanosteryl acetates) found in wool fat on analysis, plus added amounts of cholesterol and lanosteryl acetates, expressed as Gm./L.

^b Percentage of estimated quantity found on analysis

amount of the esters calculated to be present on the basis of the original analysis. The results obtained were found to agree with the estimated content of the solutions (Table V). Wool fat unsaponifiables were treated in the same manner with added amounts

of cholesterol and lanosterol. The results obtained agreed with the estimated concentrations of the solutions (Table VI).

A further test to determine the reliability of the method for estimating the amount of cholesterol and isocholesterol in wool fat was made by calculating, on the basis of molecular weight, the amounts of free sterols equivalent to the esterified sterols found

TABLE VI.—RESULTS OF ANALYSIS OF WOOL FAT UNSAPONIFIABLES PLUS ADDED AMOUNTS OF CHOLESTEROL AND LANOSTEROL

Sample	Cholesterol		Isocholesterol	
	Taken ^a	Found ^b , %	Taken ^a	Found ^b , %
152-C	0 0559	98 3	0 0374	100 4
152-D	0 0657	98 9	0 0436	100 4
154-C	0 0489	100 4	0 0322	102 3
154-D	0 0586	101 2	0 0384	99 8
Average		99 7		100 7

^a The estimated concentration of solution on basis of amount of cholesterol and isocholesterol (calculated as lanosterol) found in wool fat unsaponifiables on analysis plus added amounts of cholesterol and lanosterol, expressed as Gm./L.

^b Percentage of estimated quantity found on analysis

in wool fat. This value was compared to the amount of free sterols in wool fat estimated on the basis of total unsaponifiable from wool fat and the free sterols found in the unsaponifiable. The calculations indicate that comparable results were obtained for cholesterol by either method of estimation (Table VII). In the case of isocholesterol, the results obtained by estimation of lanosterol equivalent to the lanosteryl acetate found in whole wool fat were somewhat higher than those obtained on the basis of the free lanosterol in wool fat unsaponifiable.

TABLE VII.—COMPARISON OF RESULTS OBTAINED BY DETERMINATION OF STEROLS IN WOOL FAT DIRECTLY AND DETERMINATION OF STEROLS IN WOOL FAT UNSAPONIFIABLES

Cholesterol esters, calcd. as acetate	23 0%
Free cholesterol equiv. to acetate	20 8%
Isocholesterol esters, calcd. as lanosteryl acetate	16 5%
Free lanosterol equiv. to lano acetate	15 0%
Unsaponifiable (49.3% of wool fat)	
Cholesterol	41 6%
Equivalent amt. in whole wool fat	20 5%
Isocholesterol calcd. as lanosterol	28 1%
Equivalent amt. in whole wool fat	13 8%

CONCLUSIONS

The application of the spectrophotometric method of Luddy, *et al.* (10), in the present work was found to require modification. The difficulty in its application lies in the variable rate of development of the color complexes of cholesterol, isocholesterol, and

their esters, and in the rapid deterioration of the complexes after formation.

The rate of development of the color complexes was affected greatly by temperature variation. The earlier workers had allowed the reaction to proceed at room temperature, variations of which gave inconsistent results. The first step in this work was to control the temperature of the reaction mixture, so that the rate for each separate component would be constant. A bath temperature of 30° was selected because it was easier to maintain and the rate of color development was increased in some cases.

Luddy, *et al*, used the absorptivities of the cholesterol and lanosterol complexes for the calculation of all solutions whether they contained the free sterols or their esters. They inserted a factor in the calculation of combined cholesterol to take into account the 20% greater light absorption by the color complex of cholesterol esters as compared to the complex of free cholesterol. They did not use a factor in calculating combined ischolesterol on the assumption that light was absorbed to the same extent by the color complexes of both combined and free ischolesterol (10). On the contrary, it was found in this work that the lanosteryl acetate complex also absorbed about 20% more light than the free lanosterol complex.

The difference in rate of development of and amount of light absorption by the color complexes of the free sterols and their esters was overcome by determining the absorptivities of the complexes of cholesterol, lanosterol, and their acetates at the wavelength and time of maximum light absorption for the cholesterol complex (630 m μ) and the lanosterol complex (550 m μ). The free sterols, as in wool fat unsaponifiables, were calculated by employing the absorptivities of the cholesterol and lanosterol complexes; and the combined sterols, as in wool fat, by employing the absorptivities of the cholesteryl and lanosteryl acetate complexes. The utilization of different absorptivity values corresponding to the free sterols and their esters takes into account the difference of light absorption by the various color complexes. The variation in rate of development is compensated for by using the absorptivities at the respective times of maximum light absorption.

This procedure may be applied to the determination of combined sterols in wool fat directly, or to the determination of free sterols in wool fat unsaponifiable. The former application has the advantage that no previous treatment is necessary, but apparently this method is less reliable and also requires absorbances of unknown solutions to be observed at two different times. The determination of free

sterols in wool fat unsaponifiable, seems to be more accurate and is less difficult to run because the difference of time for maximum color development is less, and it has been shown that the use of an average time between these two maxima gives results which are consistent with those obtained by observation of absorbances at two different times.

This method is suggested for the determination of wool fat sterols in cosmetic and medicinal preparations and current investigation is being carried out along these lines.

SUMMARY

1. A modified procedure for the spectrophotometric determination of cholesterol and ischolesterol is presented. This method involves, the control of temperature during the development of the Liebermann-Burchard color complexes, the determination of absorptivities of the various sterol components at the time of maximum color development, and the use of the absorptivities of cholesteryl and lanosteryl acetate color complexes in determination of combined cholesterol and ischolesterol in wool fat.

2. This procedure can be applied to the determination of combined cholesterol and ischolesterol (as cholesteryl and lanosteryl acetates) in wool fat directly and also to the determination of free cholesterol and ischolesterol in wool fat unsaponifiables.

3. Data are presented showing results obtained on application of the method to both wool fat and wool fat unsaponifiables.

REFERENCES

- (1) Lederer, E., and Velluz, L., *Ind. parfums*, 2, 282 (1917).
- (2) Truter, E. V., "Wool Wax," Interscience Publishers Inc., New York, 1956, pp. 31-60, 160-191.
- (3) Zak, B., and Ressler, N., *Am. J. Clin. Pathol.*, 25, 133 (1955).
- (4) Pearson, S., Stern, S., and McGavack, T. H., *Anal. Chem.*, 25, 813 (1953).
- (5) Zlatkis, A., Zak, B., and Boyle, A. J., *J. Lab. and Clin. Med.*, 41, 186 (1953).
- (6) Knol, H. W., *Rec. trav. chim.*, 73, 921 (1954).
- (7) Lederer, E., and Telen, P. K., *Bull. soc. chim. biol.*, 27, 119 (1945).
- (8) Velluz, L., and Lederer, E., *ibid.*, 27, 211 (1945).
- (9) Kelsey, F. E., *J. Biol. Chem.*, 127, 15 (1939).
- (10) Luddy, F. E., Turner, A., and Scanlon, J. T., *Anal. Chem.*, 25, 1497 (1953).
- (11) Duwell, H., *ibid.*, 25, 1548 (1953).
- (12) Doree, C., and Garratt, D. C., *J. Soc. Chem. Ind. (London)*, 52, 141T (1933).

The Identification and Quantitative Analysis of Certain Quaternary Ammonium Compounds using Ultraviolet Absorption Spectra*

By M. PERNAROWSKI and L. G. CHATTEN

The ultraviolet spectra of four quaternary ammonium compounds, 1-hexadecylpyridinium chloride, *p*-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride, dodecyl-acetamido-dimethyl-benzyl ammonium chloride, and *Beta*-phenoxy-ethyl-dimethyl-dodecyl ammonium bromide, are reported. The use of the spectral characteristics of these compounds in the identification and analysis of the pure substance are discussed. The application of the ultraviolet procedure to the analysis of commercial preparations containing quaternary ammonium compounds is described.

THE INTRODUCTION of the quaternary ammonium compounds into commerce provided industry with a unique substitute for the toxic phenol-type disinfectants. Their widespread use in various industrial operations has presented to the analyst a challenging problem in separation, identification, and quantitative analysis in such unrelated substances as eggs (1), milk (2), shrimp (3), and beer (4). There have been two basic approaches to the analysis of quaternary ammonium compounds. The first involves the preparation of an insoluble derivative of the quaternary ammonium compound and the second involves titration of some portion of the molecular entity.

Substituted ammonium compounds are capable of reacting with certain anions to form salts which are either insoluble or which may be extracted from the reacting phase by means of organic solvents. Examples of such insoluble salts are the ferricyanides, ferrocyanides, silicotungstates, dichromates, and permanganates. Wilson (4) has described a procedure involving the preparation of the ferricyanide salt of the quaternary ammonium compound. Tilson, Eisenberger, and Wilson (5) showed that these basic type compounds were capable of reacting with ammonium reineckate. By preparing these reineckates and determining their optical crystallographic properties, they were able to identify a large number of quaternary ammonium compounds. By far the most common approach to the analysis of this type of compound has been the preparation of a complex using indicators such as bromophenol blue or bromocresol green (1, 3, 6-12). The procedure involves the coupling of the quaternary ammonium compound with the indicator and determining its color intensity in a suitable photoelectric colorimeter.

Several volumetric procedures have been reported for the determination of quaternary ammonium compounds. Carkhuff and Boyd (13) titrated 1-hexadecylpyridinium chloride using sodium lauryl sulphate solution and methyl yellow indicator.

Pifer and Woolish (14) showed the quaternary ammonium compounds could be titrated in non-aqueous solvents with acetous perchloric acid. Caswell (15) reported that the halogen portion of the molecule could be titrated with silver nitrate solution either electrometrically or with dichloro-fluorescein indicator solution.

Experience in this laboratory with ultraviolet absorption spectroscopy appeared to indicate that the spectra of these compounds might offer a means of both qualitative and quantitative analysis for various commercial preparations. While this investigation was in progress, a preliminary report on the absorption spectra of two of the quaternary ammonium compounds discussed in this paper was published by Caswell (16).

EXPERIMENTAL

This study included the following compounds: 1-hexadecylpyridinium chloride (Cecpryn® chloride; cetylpyridinium chloride); *p*-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride (Phemerol® chloride; benzethonium chloride; phenicide; Hyamine 1622®); dodecyl-acetamido-dimethyl-benzyl ammonium chloride (Nopecide K®); and *Beta*-phenoxy-ethyl-dimethyl-dodecyl ammonium bromide (Bradosol®).¹

The pure crystalline quaternary ammonium compounds were analysed by titration in a nonaqueous solvent prior to use. A sample of the quaternary ammonium compound was weighed, dissolved in 50 ml. chloroform and 2 ml. of 6% mercuric acetate in glacial acetic acid and titrated with 0.05 *N* perchloric

* Received August 5, 1957 from the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.

¹ Synonyms taken from the manufacturer's literature or *The Merck Index*.

acid in dioxane. Methyl red (0.1% solution in glacial acetic acid) was found superior to crystal violet in these titrations and was, therefore, used in all the analysis. The solvent system described by Pifer and Woolish (14) was found to be equally satisfactory.

All preliminary ultraviolet spectra were obtained by scanning a suitable solution from 200 $m\mu$ to 300 $m\mu$ on a Cary recording spectrophotometer. After maximum and minimum points had been established, analyses of solutions of quaternary ammonium compounds were conducted using a Beckman DU spectrophotometer.

(a) **1-Hexadecylpyridinium chloride.**—The specific extinction coefficient (a_s) of 1-hexadecylpyridinium chloride was calculated from the absorbance (A_s) value observed for a solution containing 0.025 mg. per ml. One cm. cells were used in all determinations. This value of a_s was used to determine the concentrations of all subsequent solutions prepared in this laboratory. A series of solutions were prepared to contain between 0.01 to 0.05 mg. of the quaternary ammonium compound per ml. From calculations of the molar extinction coefficient (a_m) and from a plot of A_s against the concentration, it would appear that Beer's Law is obeyed. Table I records the results obtained.

Figure 1 shows the ultraviolet absorption spectrum of 1-hexadecylpyridinium chloride. The ultraviolet characteristics of this compound are recorded in Table II. The value of a_m at 259 $m\mu$, the point of peak absorbance, was approximately 4280. This figure represents the average value found for ten determinations representing two separate sets of solutions. An approximate error of 1% was noted between these two sets of determinations.

(b) ***p*-Diisobutyl - phenoxy - ethoxy - dimethylbenzyl Ammonium Chloride.**—The procedure followed was essentially the same as that reported in (a). The solutions studied had concentrations ranging from 0.08 to 0.40 mg. of the quaternary ammonium compound per ml. Both the molar extinction coefficient and the absorbance-concentration graph indicated that Beer's Law was being obeyed. The value of a_m at 269.5 $m\mu$, the point of peak absorbance, showed only slight variation over the whole absorbance range of the Beckman DU spectrophotometer. This is shown numerically by the low standard deviation reported in Table II. Figure 2 shows the ultra-

violet spectrum of *p*-diisobutyl-phenoxy-ethoxy-dimethylbenzyl ammonium chloride.

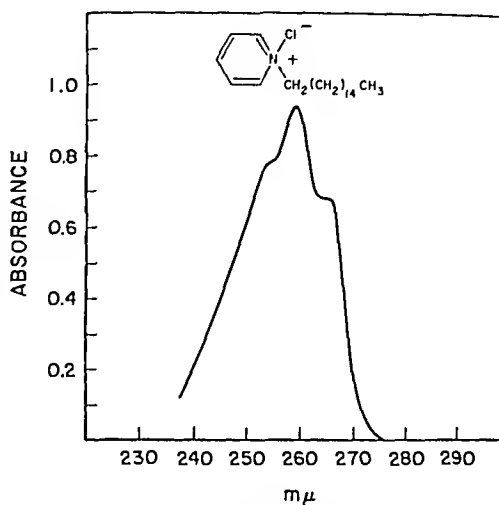


Fig. 1.—The ultraviolet spectrum of 1-hexadecylpyridinium chloride.

TABLE I.—RECOVERY OF QUATERNARY AMMONIUM COMPOUNDS FROM PREPARATIONS OF KNOWN COMPOSITION

Quaternary Ammonium Compound	Purity by Non-aqueous Titration, %	Recovery Spectrophotometric, %	Standard Deviation $n = 9$
1-Hexadecylpyridinium chloride	99.8	100.4	+1.1
β -Phenoxy-ethyl-dimethyl-dodecyl Ammonium Bromide	100.0	101.0	± 1.7
Isododecyl-acetamido-dimethyl-benzyl Ammonium Chloride	100.0	99.1	± 2.0
<i>p</i> -Diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl Ammonium Chloride	99.4	100.0	± 1.0

TABLE II.—ULTRAVIOLET ABSORPTION CHARACTERISTICS OF FOUR QUATERNARY AMMONIUM COMPOUNDS

Quaternary Ammonium Compound	Maxima	Minima	Shoulders	Optimum Concentration mg./ml.	a_m
1-Hexadecylpyridinium chloride	<u>259</u>	...	254-255 264-265	0.04	4280 $\pm 0.7\%$
Beta-Phenoxy-ethyl-dimethyl-dodecyl Ammonium Bromide	268.5 <u>274.5</u>	272.5	263-264	0.20	1285 $\pm 1.5\%$
Isododecyl-acetamido-dimethyl-benzyl Ammonium Chloride	267.5 <u>269</u>	267	252-253	0.50	425 $\pm 1.9\%$
<i>p</i> -Diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl Ammonium Chloride	269.5 <u>264</u> 275	265 272	257-259 280-281	0.15	1430 $\pm 0.6\%$

^a Values of a_m reported are for the wavelengths underlined. Standard deviations are reported immediately after the a_m value.

This quaternary ammonium compound was one of the two reported in this paper that had been studied by Caswell (16). Table II and Fig. 2 show that this compound has three points of peak absorption. Caswell reported similar observations, indicating that ultraviolet maximum occurred at 264 $m\mu$, 269 $m\mu$, and 274 $m\mu$. Within experimental limits, these results agree well with those observed in this laboratory.

(c) **Beta-Phenoxy-ethyl-dimethyl-dodecyl Ammonium Bromide.**—Solutions were prepared and diluted so that the concentration of the quaternary ammonium compound varied from 0.05 to 0.30 mg. per ml. Absorbancy-concentration graphs and the a_m values at 268.5 indicated that Beer's Law was being obeyed. There appeared to be a slight variation between two sets of solutions. The average a_m value for one set was approximately 1275 while that observed for the other set was approximately 1300. In both cases, however, the standard deviation indicated that either answer was within the experimental limits set by any one series of solutions. The average value is reported in Table II along with other ultraviolet characteristics of this compound. Figure 2 shows the spectral curve for β -phenoxy-ethyl-dimethyl-dodecyl ammonium bromide.

(d) **Dodecyl-acetamido-dimethyl-benzyl Ammonium Chloride.**—The specific extinction coefficient for this compound was determined in the usual way. The concentrations of the solutions varied from 0.1 to 1.0 mg. of the quaternary ammonium compound per ml. Graphical evaluation and a_m values at 263 $m\mu$ indicated that Beer's Law was being obeyed. There appeared to be a slight difference in a_m values in those cases where the concentration of the quaternary ammonium compound fell to approximately 0.1 mg. per ml. and exceeded 1.0 mg. per ml. These slight deviations may be expected since the concentrations represent very high and very low absorbancy values. These deviations represent an approximate error of 2% in calculation of percentage recovery from a known preparation.

The ultraviolet characteristics of dodecyl-acetamido-dimethyl-benzyl ammonium chloride are reported in Table II. An ultraviolet spectrum of this compound is shown in Fig. 2. This quaternary ammonium compound, although not reported in tabular form by Caswell (16), was discussed in the text of the paper. He reported that this compound showed the same ultraviolet characteristics as did alkyl-dimethyl-benzyl ammonium chloride and cetyl-dimethyl-benzyl ammonium chloride. Evidently the absorption appears to be characteristic of the benzyl group. There was excellent agreement between the results obtained in this laboratory and those reported by Caswell.

The following general procedure is recommended for estimating the concentration of compounds of the type mentioned in this paper.

Weigh accurately the quaternary ammonium compound and dissolved in sufficient water to make the concentration such that it will be suitable for ultraviolet examination. Dilution of the original solution by the aliquot procedure may be required. Since many quaternary ammonium compounds are marketed in an aqueous solution, dilution to the required concentration may be the only step required. Transfer a portion of the solution to a 1-cm. cell, and using water as the blank, scan the solution

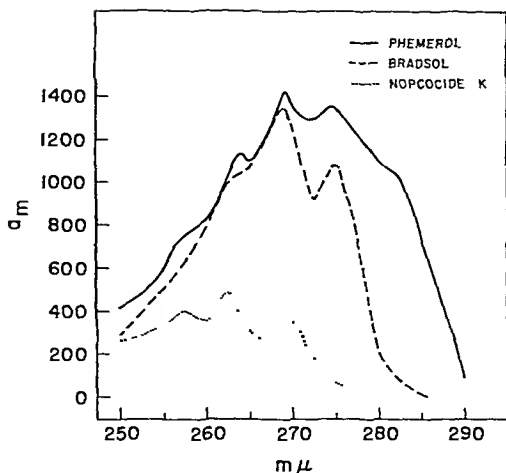


Fig. 2.—The ultraviolet spectra of three quaternary ammonium compounds.

on the Cary recording spectrophotometer from 220 to 300 $m\mu$. Determine the absorbancy from the chart record or by using a Beckman DU spectrophotometer set at the required wave length. By observing the characteristics of the spectra and from the value of a_m obtained for the compound, the quaternary ammonium compound may be identified with some degree of certainty. For the purposes of this paper, the a_m value at peak absorption has been reported. Values for a_m at any other absorption maxima or minima may be calculated for the purposes of identification. Where the original concentration of the quaternary ammonium compound is unknown, a standard should be run under identical conditions to establish a_s values.

DISCUSSION

There appears to be a certain amount of similarity between three of the four spectra. Dodecyl-acetamido-dimethyl-dodecyl ammonium bromide, and *p*-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride both show an absorption maxima at 269 $m\mu$. At this point, only a_m values would serve to distinguish the compounds. In addition, the first and last compounds also show absorption maxima at approximately 264 $m\mu$. Similarly, the second and third compounds show similarity in the region 274 $m\mu$. The actual differences are best seen in Fig. 2. In this figure, a_m values have been plotted against the wave length. It will be noted that the point of greatest difference between β -phenoxy-ethyl-dimethyl-dodecyl ammonium bromide and *p*-diisobutyl-phenoxy-ethyl-dimethyl-benzyl ammonium chloride lies at 275 $m\mu$. This point is, therefore, a key point in positive identification of the quaternary ammonium compound. Experimental errors may give inconclusive results at 269 $m\mu$ or at 263 to 264 $m\mu$. No difficulty should be experienced in distinguishing between dodecyl-acetamido-dimethyl-benzyl ammonium chloride and the above mentioned compounds. 1-Hexadecylpyridinium chloride presents no problem because its peak absorption occurs at much lower wavelengths.

The method described was applied to several pharmaceutical preparations containing quaternary ammonium compounds. With commercial preparations, it was found that the precision of the method was quite good. One of the major difficulties with pharmaceutical preparations was the presence of dyes in either the aqueous solutions or the tinctures. These, if present in high concentration, interfered with the analysis. The characteristics of the absorption curve, in the cases studied, were not altered and, therefore, it was still possible to identify the quaternary ammonium compound. In one commercial preparation analyzed, results obtained were approximately 30% higher than that stated on the label but the quaternary ammonium compound was easily identified as *p*-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride.

Caswell (16) reported that interfering substances may be removed by acidification of the solution of the quaternary ammonium compound and extraction of the substance with ether. Another possible approach involves the use of the base line technique. It was found in this laboratory that incorporation of the dye present in the solution into the blank gave satisfactory results. If the dye concentration is not high, the color intensity is low and the quaternary ammonium compound can be determined. The results obtained for a typical preparation are shown in Table III. The results in this Table indicate that an error of 10% in the dye concentration will still lead to satisfactory results. Usually

TABLE III.—ABSORBANCY VALUES FOR COLORED AND UNCOLORED SOLUTIONS OF *p*-DIISOBUTYL-PHENOXO-ETHOXY-ETHYL-DIMETHYL-BENZYL AMMONIUM CHLORIDE

Deter- mina- tion	Mg./ml of Com- pound	Mg. Dye/ l. of Solution	Mg. Dye/ l. of Blank	A.
I	0.12	0.00	0.00	0.368 0.371
II	0.12	5.00	5.00	0.363 0.380
III	0.12	5.00	4.50	0.374 0.372

a calibration curve was obtained for the dye using a Cary recording spectrophotometer and the dye concentration in the commercial preparation was thus determined. This was then utilized in preparing a proper dye concentration in the blank. The above method was found to be very satisfactory for laboratory preparations and for several commercial preparations. Two commercial preparations could not be analyzed in this way because of high dye concentration (of the order of 40 mg. per liter in the final dilution).

Not only is the presence of dye a limiting factor, but also the presence of alcohol will cause difficulty. In this case, the problem is less complex and with proper dilution (usually with 95% alcohol) Beer's Law is valid. This was shown to be the case for

p-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride, 1-hexadecylpyridinium chloride, and dodecyl-acetamido-dimethyl-benzyl ammonium chloride. In the presence of alcohol, however, peak absorbancies reported in water are not valid. These must be established on the basis of the preparation being analyzed. Slight shifts in peak absorption points and changes in a_m values were noted for the three preparations listed above. As an example, *p*-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride showed four absorption maxima ($\lambda_{\text{max.}} = 263, 269, 276, 283 \text{ m}\mu$) in approximately 50% ethanol. It would appear that the shoulder at 280–281 $\text{m}\mu$ in aqueous solution becomes a peak in alcoholic solution. The differences for the other quaternary ammonium compounds were found to be less pronounced. Again, the first of these three quaternary ammonium compounds listed above was studied in a colored, aqueous alcohol solution. By using the technique described above, it was found that a linear relationship existed between the absorbancy of the solution and its concentration.

In recent months, Crane (17) showed that sodium tetraphenylboron formed an insoluble derivative with certain quaternary ammonium compounds. This is being investigated in this laboratory at the present time. Preliminary results show that not only quaternary ammonium compounds but also other compounds having a basic nitrogen may be separated from mixtures of substances not having this property.

The most plausible approach to commercial preparations containing quaternary ammonium compounds would appear to be extraction of the substances into a nonaqueous solvent and subsequent titration with perchloric acid in dioxane. This approach was investigated and found to give precise results. However, the accuracy of the method was not as good as expected. Only about 95% of the active ingredient could be extracted using conventional techniques.

REFERENCES

- (1) Wilson, J. B., *J. Assoc. Offic. Agr. Chemists*, 33, 666 (1950).
- (2) Miller, D. D., and Elleker, P. R., *J. Dairy Sci.*, 34, 273 (1951).
- (3) Wilson, J. B., *J. Assoc. Offic. Agr. Chemists*, 33, 670 (1950).
- (4) Wilson, J. B., *ibid.*, 31, 108 (1948).
- (5) Wilson, A. H., Eisenberger, Wm. V., and Wilson, J. B., *ibid.*, 35, 459 (1952).
- (6) Ballard, C. W., Isaacs, J., and Scott, P. G. W., *J. Pharm. Pharmacol.*, 6, 971 (1954).
- (7) Colichman, E. L., *Anal. Chem.*, 19, 430 (1947).
- (8) Harris, T. H., *J. Assoc. Offic. Agr. Chemists*, 29, 310 (1946).
- (9) Wilson, J. B., *ibid.*, 29, 311 (1951).
- (10) Colichman, E. L., *J. Am. Chem. Soc.*, 73, 3385 (1951).
- (11) Fogh, J., Rasmussen, P. O. H., and Skadhauge, K., *Anal. Chem.*, 26, 392 (1954).
- (12) Auerbach, M. R., *Ind. Eng. Chem., Anal. Ed.*, 16, 739 (1944).
- (13) Crnkluft, E. D., and Boyd, Wm. F., *THIS JOURNAL*, 43, 240 (1954).
- (14) Pifer, C. W., and Wollish, E. G., *Anal. Chem.*, 24, 300 (1952).
- (15) Caswell, R. L., *J. Assoc. Offic. Agr. Chemists*, 34, 675 (1951).
- (16) Caswell, R. L., *ibid.*, 38, 304 (1955).
- (17) Crane, F. E., Jr., *Anal. Chem.*, 28, 1794 (1956).

The Assay of Procaine Penicillin in Sesame Oil by Optical Rotation*

By B. A. PAPPAS and J. D. DUERR

The extraction of Procaine Penicillin from a suspension containing sesame oil and aluminum monostearate using chloroform and formamide with subsequent assay by optical rotation is described. It is also compared to an assay in present use involving the solution of the suspension in hot chloroform and subsequent assay by optical rotation.

OF NECESSITY CONTROL PROCEDURES in the pharmaceutical industry must be rapid and yet accurate. Most of the assays have been designed for quick determination and one of the quickest is optical rotation. Penicillin salts in general can be assayed by optical rotation after finding a suitable solvent. This method, for control purposes while formulating and filling, is very rapid and accurate.

When the formulation contains a vehicle that is difficult to remove from the active material, or contributes cloudiness or haziness to the solution, the accuracy of the assay procedure invariably suffers. Filtering almost any solution containing a solvent commonly used for the determination of optical rotation will result in erratic results, usually on the high side. The solvents commonly used for the assay of procaine penicillin when applied to oil suspensions containing aluminum monostearate either did not dissolve the procaine penicillin or gave extremely hazy solutions even after heating and filtering. The solvent generally used is chloroform. The extracts are usually heated and filtered. The present experiments were undertaken in order to find a solvent or solvent mixture that would dissolve the Procaine Penicillin, free the oil and aluminum monostearate and leave a clear solution ready for measurement by optical rotation. At the same time the intention was to eliminate the deviation between filtered and nonfiltered solutions, the deviation between filtered samples themselves, and the deviation due to different heating conditions used to dissolve the sample suspension.

EXPERIMENTAL

Assay of a Standard Suspension of Procaine Penicillin in Oil.—A suspension was made to contain exactly 300,000 units of procaine penicillin per ml. with a specific gravity of 1.0. The vehicle was 2%

aluminum monostearate in sesame oil. Five individual 3-Gm. samples of suspension were transferred to separatory funnels. Fifty ml. of formamide (Special Decolorized Charles Pfizer Grade manufactured by Matheson, Coleman & Bell) and 25 ml. of chloroform were added to each separatory funnel and the mixture was shaken for approximately thirty seconds. The two phases formed were then allowed to separate completely and the bottom formamide layer was optically clear. Measurements of the optical rotation of the formamide layer were made in a 1-decimeter tube using the sodium D line illumination. The results are shown in Table I.

TABLE I.—ANGLE OF ROTATION OF FIVE 300,000 UNITS/CC. STANDARDS

Sample	Total Units	Angle°
1	900,000	2.64
2	900,000	2.65
3	900,000	2.64
4	900,000	2.63
5	900,000	2.64

The conclusion, therefore, was that an average angle of rotation of 2.64° would be obtained when 900,000 units of the above standard procaine penicillin in sesame oil were treated using this procedure.

The Effect of Varying the Concentration of Sesame Oil and Aluminum Monostearate.—Samples of the standard procaine penicillin containing 900,000 units each were transferred to separatory funnels. Diluent containing sesame oil and aluminum monostearate was added to each sample. The volume of diluent ranged from 1.0–3.0 ml. The assay procedure was repeated and the formamide solutions were read in a 2-decimeter tube. The effect of varying concentrations of aluminum monostearate and sesame oil on the angle of rotation is shown in Table II. We concluded, therefore, from the results

TABLE II.—EFFECT OF CONCENTRATION OF SESAME OIL AND ALUMINUM MONOSTEARATE ON ANGLE OF ROTATION

Units Procaine Penicillin	Vol of Added Oil and A.M.S., cc.	Angle of Rotation
900,000	1	5.15
900,000	1.5	5.20
900,000	2.0	5.20
900,000	3.0	5.22

in Table II that small variations in concentration of diluent had no serious effect on the optical rotation since the difference between the highest and lowest concentration was only 1.34%.

Reproducibility of Assay Using Different Weights of Sample.—Samples of a standard suspension of 300,000 units/ml. ranging from 0.9306 Gm. to

* Received June 26, 1957 from Chas. Pfizer & Co. Inc., Process Development Laboratory, Pharmaceutical Production and Packaging Department, Brooklyn, N. Y.

TABLE III—REPRODUCIBILITY OF ASSAY USING DIFFERENT WEIGHTS OF SAMPLE^a

Wt of Sample, Gm	Units Introduced	Angle of Rotation	Units Recovered	Units/ml	Deviation in Thousands
0.9306	279,180	0.80	272,727	293,000	-6.6
1.5519	465,570	1.40	477,272	307,540	+7.8
1.7596	527,880	1.54	525,000	298,400	-1.3
2.5786	773,580	2.25	751,894	291,500	-8.2
2.9130	873,900	2.55	869,318	298,400	-1.3
3.0000	900,000	2.64	900,000	300,000	0
3.0000	900,000	2.65	903,000	301,000	+1.3
3.0000	900,000	2.61	900,000	300,000	0
3.0000	900,000	2.63	897,000	299,000	-0.7
3.2450	973,500	2.88	981,000	302,000	+2.3
3.7358	1,120,740	3.34	1,138,636	304,600	+4.9
3.8280	1,148,400	3.38	1,152,272	301,000	+1.3

^a Theoretical Potency = 300,000 units/ml. Average Assay = 299,708 units/ml. Standard Deviation of Assay = 4,175 units/ml.

3.8280 Gm. were assayed using the same procedure. The standard procaine in oil suspension was prepared as follows: An amount of bulk procaine penicillin of known potency was mixed with sesame oil containing 2% aluminum monostearate to give a theoretical potency of 300,000 u/ml. The standard average angle of 2.64° was used to calculate total units per ml. recovered by assay. The results are shown in Table III.

Assay of an Unknown.—Five gallons of a production lot were set aside in the laboratory and used as an unknown before the potency was confirmed. All subsequent reference to an unknown pertains to this 5-gal. sample. Three samples were assayed and their potency calculated using an optical rotation of 2.61° equivalent to 300,000 u/ml. The results are shown in Table IV.

TABLE IV—ASSAY OF UNKNOWN

Sample	Theoretical Units Introduced	Units Recovered by Assay
1	301,000	301,200
2	710,000	718,000
3	886,000	889,000

Reproducibility of Assay.—Twenty samples from the unknown mixture of procaine Penicillin sesame oil were assayed by two technicians using 3.0-Gm. samples. The standard angle of 2.64° was used to calculate units per ml. of procaine penicillin. The results are shown in Table V.

Biological Assay of the Same Unknown.—The unknown used in the previous experiments was submitted for the microbiological assay of procaine penicillin. The results are shown in Table VI.

Assay Using Chloroform as the Solvent.—Ten samples were taken from the same unknown and each transferred to a 50-ml. volumetric flask. The suspension was then shaken with chloroform and the flask brought to volume with chloroform. A very cloudy solution developed. The flasks were then immersed in hot water until the solution cleared.

TABLE V—COMPOSITE OF RESULTS OF TWO TECHNICIANS^a

Sample	Angle of Rotation	Potency units/ml	Deviation in Thousands	(Deviation in Thousands) ²
1	2.60	295,400	+3.5	12.25
2	2.55	289,700	-2.2	4.84
3	2.57	292,000	+0.1	0.01
4	2.59	294,300	+2.4	5.76
5	2.57	292,000	+0.1	0.01
6	2.58	293,200	+1.3	1.69
7	2.53	287,500	-4.2	17.64
8	2.57	292,000	+0.1	0.01
9	2.58	293,200	+1.3	1.69
10	2.56	290,900	-1.0	1.00
11	2.60	295,400	+3.5	12.25
12	2.56	290,900	-1.0	1.00
13	2.56	290,900	-1.0	1.00
14	2.56	290,900	-1.0	1.00
15	2.56	290,900	-1.0	1.00
16	2.56	290,900	-1.0	1.00
17	2.57	292,000	+0.1	0.01
18	2.57	292,000	+0.1	0.01
19	2.56	290,900	-1.0	1.00
20	2.56	290,900	-1.0	1.00

^a Average Potency = 291,900 units/ml. Standard Deviation = 1,790 units/ml. 2 σ Limits = 288,320 to 295,480 units/ml. 95% Confidence interval observed value $\pm 1.22\%$. 99% Confidence interval = observed value $\pm 1.84\%$.

TABLE VI.—RESULTS OF BIOLOGICAL ASSAY^a

Sample	Potency Units/ml	Deviation in Thousands	(Deviation in Thousands) ²
1	285,000	+1	1
2	285,000	+1	1
3	280,000	-4	16
4	295,000	+11	121
5	280,000	-4	16
6	290,000	+6	36
7	300,000	+16	256
8	280,000	-4	16
9	280,000	-4	16
10	285,000	+1	1

^a Average Potency = 281,000 units/ml. Standard Deviation = 6,900 units/ml. 2 σ Limits = 270,200 to 297,800 units/ml. 95% Confidence interval observed value $\pm 2.1\%$. 99% Confidence interval observed value $\pm 2.8\%$.

TABLE VII.—POTENCY OF UNKNOWN PROCAINE PENICILLIN IN SESAME OIL USING CHCl_3 AS SOLVENT AND FILTERED BEFORE READING

Sample	Potency Units/ml	Deviation	(Deviation in Thousands) ²
1	311,592	+ 2 3	5 29
2	308,210	- 1 1	1.21
3	314,874	+ 5 6	31 40
4	316,540	+ 7 2	51.80
5	321,538	+12 2	148 84
6	304,878	- 4 4	19 40
7	304,878	- 4 4	19 40
8	304,878	- 4 4	19 40
9	301,546	- 7 8	60 84
10	308,210	- 1 1	1 21
11	304,878	- 4 4	19 40

Average Potency = 309,275 units/ml, Standard Deviation = 5,850 units/ml
 2σ Limits = 297,575 to 320,975 units/ml, 95% Confidence interval = Observed value $\pm 3.71\%$, 99% Confidence interval = Observed value $\pm 5.67\%$

TABLE VIII.—POTENCY OF UNKNOWN PROCAINE PENICILLIN IN SESAME OIL USING CHCl_3 AS SOLVENT AND READ UNFILTERED^a

Sample	Potency units/ml	Deviation in Thousands	(Deviation in Thousands) ²
1	299,880	-3 0	9 00
2	301,546	-1 4	1 96
3	308,210	+6 3	3 97
4	306,544	+3 6	3 97
5	304,878	+2 0	4 00
6	299,880	-3 0	9 00
7	299,880	-3 0	9 00
8	301,546	-1 4	1 96
9	301,546	-1 4	1 96
10	299,880	-3 0	9 00
11			

^a This particular sample of procaine penicillin in oil was clear enough to read without having to resort to approximation on the part of the technician. Average Potency = 302,905 units/ml

Standard Deviation = 2,240 units/ml
 2σ Limits = 298,425 to 307,385 units/ml, 95% Confidence = 1.47% 99% Confidence = 2.21%

$(\frac{1}{2} \times \text{Volume of Solution} \times \text{SG} \times 1008 \text{ Units/mg}) / ([\alpha]_D^{25} \times \text{Wt}) = \text{Units/ml}$
 Procaine Penicillin where $[\alpha]_D^{25} = 102$; SG = 1.0; Wt = 3.0 Gm, Vol = 50 ml and Units/mg Procaine Penicillin = 1008. The results are shown in Table VII and VIII

SUMMARY

1. The potency of procaine penicillin in sesame oil suspensions can be determined by using formamide and chloroform for extraction and subsequent measurement of optical rotation. The average potency comes closer to the average reported by the microbiological method than the result obtained by determining the potency by optical rotation in straight heated chloroform.

2. The average deviation and standard deviation of this assay is far lower than those obtained using either the microbiological assay or the chloroform assay.

3. The results show that the difference obtained between filtered and unfiltered chloroform solutions is very great. Average and standard deviations are higher when the chloroform solution is filtered.¹

4. Extraction using formamide and chloroform gives results that approach theoretical potencies of procaine penicillin in sesame oil suspensions much closer than those obtained by either microbiological assay or the use of heated chloroform.

5. Since one cannot know in advance whether the chloroform solution has to be filtered before reading the optical rotation of the solution, it would seem advisable to adopt a method that

TABLE IX.—A COMPARISON OF DEVIATIONS AND PER CENT CONFIDENCE OF THE THREE METHODS OF ASSAY

Methods	Average Potency in units/ml	Standard Deviation in units/ml	2σ limits in units/ml	95% Confidence interval	99% Confidence interval
Formamide chloroform	291,900	1,790	295,480 288,320	± 1.22	± 1.84
Chloroform (filtered)	309,275	5,850	320,975 297,575	± 3.71	± 5.67
Bioassay	284,000	6,900	297,800 270,200	± 5.21	± 7.28
Chloroform (unfiltered)	302,905	2,240	307,385 298,425	± 1.47	± 2.21

Care was taken that the chloroform solution was not brought to the boiling point. The solutions were then cooled until they returned to a 50-ml volume. The angle of rotation of each solution was read using the sodium D illumination both filtered and unfiltered. The potency was determined using the following formula:

eliminates the necessity of filtering at any time.

¹ Much weight must be given to conclusion 3. The effect of filtration is not made clear by separate analysis of filtered and unfiltered results. In reality the effect of filtration is randomly distributed since the practice of necessity is to filter unreadable samples. This practice leads to very large deviations as can readily be seen from casual inspection of the separate results.

The Effects of Certain Carbon Sources on The Growth of *Claviceps litoralis**

By W. T. GLOOR, Jr.† and H. W. YOUNGKEN, Jr.‡

Comparative growth and dry weight yields of *Claviceps litoralis*, Kawatani, grown in synthetic media with nine different carbon sources are reported. A five per cent mannitol concentration, which served as the carbon source for mycelial growth, produced best results. Glucose, mannose and levulose produced good growth and weight; whereas maltose, sorbitol, glycerol, sorbose, and xylose gave poor weight yields.

litoralis, Kawatani, grown on synthetic media. This report describes the results obtained by using nine different carbon sources.

EXPERIMENTAL

Culture Method.—Previous work on the growth studies of *C. litoralis*, Kawatani (13, 14) indicated that maltose might not be as satisfactory a carbon source as other compounds. Therefore, in preliminary work of this study 5% mannitol was used as the carbon source in a modified yeast extract-peptone medium (5) described in Table I. Mannitol produced better growth results during preliminary studies. This alcohol has also been isolated from ergot sclerotia both in free form and from *Claviceps* in, a trisaccharide occurring in the sclerotia.

TABLE I—MODIFIED YEAST EXTRACT-PEPTONE MEDIUM

Yeast extract	12.50 Gm
Peptone	1.25 Gm
MgSO ₄ 7H ₂ O	1.25 Gm
KH ₂ PO ₄	2.50 Gm
Mannitol	50.00 Gm
Distilled Water q s ad	1.00 L

Eight other carbon compounds, namely, sorbose, inosine, levulose, glucose, maltose, sorbitol, xylose, and glycerol were therefore selected and used in a molar strength corresponding to a 5 per cent mannitol solution (0.275 M). Each compound was made up into a separate medium using the same amounts of peptone, yeast extract, magnesium sulfate, and potassium phosphate as described in Table I.

Erlenmeyer flasks of 500 ml capacity were employed as culture flasks with each flask containing 100 ml of medium. Five milliliters of a mycelium suspension were inoculated into each flask of test medium.

The inoculum was prepared by first inoculating the fungus, which had been maintained on agar slants, into yeast extract-peptone medium containing 5% mannitol. This preparation was allowed to grow on a reciprocal laboratory shaker operating at two hundred 1 1/2-inch strokes per minute. At the end of four weeks the contents of the flask were poured into a sterilized steel Waring Blendor cup and blended into a fine even suspension. Five milliliters of the suspension were inoculated into each flask of test medium after which the flasks were placed on the mechanical shaker. After a 15 day growth period, (see Fig. 1) the flasks were harvested by filtering the growth through tared sintered glass crucibles and thoroughly washed with distilled water to remove all adhering constituents of the medium. The growth was dried as much as possible using a Buchner flask and vacuum and then placed in a laboratory lyophilizer to remove com-

GROWTH OF THE ERGOT FUNGUS *Claviceps purpurea* (Fries) Tulasne has been accomplished on artificial culture under various conditions by numerous workers (1-10). However the growth obtained under these conditions produced either no alkaloids or insignificant amounts of them when compared with that produced in the natural sclerotium of *Claviceps*. Bonns (1) and Kirehloff (2) reported the formation of "sclerotial tissue" in artificial cultures but this result was questioned by McCrea (3) who contended it was not true sclerotium but a "pseudosclerotium". Abe (9) suggests that pigmented sclerotial tissue is a necessary factor for the biosynthesis of ergot alkaloids in culture. Abe's contention has been in part supported by Sun and Youngken (12) and by Loo and Lewis (11) using two different experimental approaches.

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The amounts of mycelial growth which were obtained using glucose, mannose, and levulose as the individual carbon sources are comparable to those obtained with mannitol. However, fungal tissues grown on these three carbohydrates were a light brownish color instead of the purplish brown color which was produced by mycelia grown on mannitol. Although some fatty material was also produced by the organism growing on these three sugars, it did not compare quantitatively with the amount of lipid material produced by the *Claviceps litoralis* grown on mannitol.

Maltose as a carbon source produced only half as much growth as was produced by the control, mannitol. This was to be expected as this fact was shown by previous work with this particular organism (12). The remaining four carbon compounds, sorbitol, glycerol, sorbose, and xylose, could be considered very poor carbon sources under the experimental conditions used. The mycelium pellets grown on maltose were a dirty brown color while the pellets grown on the latter four compounds were small, tough, dark brown pellets. The production of lipid material by the tissues grown on the above five carbon sources was also noticeably small by comparison.



Fig. 1.—Typical mycelial pellets of *Claviceps litoralis* grown under shaking conditions on 5 per cent mannitol medium for fifteen days. (X1.)

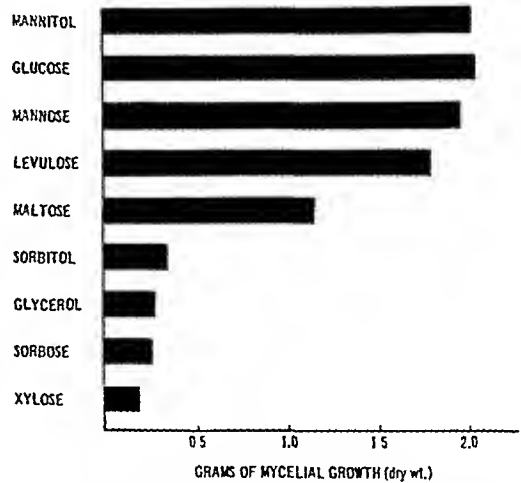


Fig. 2.—Comparison of dry weights of *Claviceps litoralis* mycelial growth on different carbohydrates

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Test Carbohydrate	Concentration, % ^a	No of Flasks	Av. Dry Wt., Mycelium/Flask, Gm.	Standard Deviation of Mean ^b	Relative, Dry Wt. of Growth, %
Mannitol ^c	5	6	2.0120	±0.0566	100
Glucose	5	7	2.0174	±0.0239	100.27
Mannose	5	7	1.9404	±0.0413	96.44
Levulose	5	7	1.7975	±0.0391	89.34
Maltose	10	7	1.1397	±0.0289	56.65
Sorbitol	5	6	0.3343	±0.0186	16.62
Glycerol	2.5	6	0.2722	±0.0109	13.53
Sorbose	5	7	0.2583	±0.0045	12.84
Xylose	4	7	0.1602	±0.0088	7.96

^a Calculated on the molar concentration of 5 per cent mannitol solution (0.275 M).

^b Standard deviation of the mean = $\pm \sqrt{\frac{\sum d^2}{n + (n - 1)}}$

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The Effects of Certain Carbon Sources on The Growth of *Claviceps litoralis**

By W. T. GLOOR, Jr.† and H. W. YOUNGKEN, Jr.‡

Comparative growth and dry weight yields of *Claviceps litoralis*, Kawatani, grown in synthetic media with nine different carbon sources are reported. A five per cent mannitol concentration, which served as the carbon source for mycelial growth, produced best results. Glucose, mannose and levulose produced good growth and weight, whereas maltose, sorbitol, glycerol, sorbose, and xylose gave poor weight yields

litoralis, Kawatani, grown on synthetic media. This report describes the results obtained by using nine different carbon sources

EXPERIMENTAL

Culture Method.—Previous work on the growth studies of *C. litoralis*, Kawatani (13, 14) indicated that maltose might not be as satisfactory a carbon source as other compounds. Therefore, in preliminary work of this study 5% mannitol was used as the carbon source in a modified yeast extract peptone medium (5) described in Table I. Mannitol produced better growth results during preliminary studies. This alcohol has also been isolated from ergot sclerotia both in free form and from *Claviceps* in, a trisaccharide occurring in the sclerotia

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TABLE I—MODIFIED YEAST EXTRACT PEPTONE MEDIUM

Yeast extract	12.50 Gm
Peptone	1.25 Gm
MgSO ₄ 7H ₂ O	1.25 Gm
KH ₂ PO ₄	2.50 Gm
Mannitol	50.00 Gm
Distilled Water q s ad	1.00 L

Eight other carbon compounds, namely, sorbose, mannose, levulose, glucose, maltose, sorbitol, xylose, and glycerol were therefore selected and used in a molar strength corresponding to a 5 per cent mannitol solution (0.275 M). Each compound was made up into a separate medium using the same amounts of peptone, yeast extract, magnesium sulfate, and potassium phosphate as described in Table I.

Erlenmeyer flasks of 500 ml capacity were employed as culture flasks with each flask containing 100 ml of medium. Five milliliters of a mycelium suspension were inoculated into each flask of test medium.

The inoculum was prepared by first inoculating the fungus, which had been maintained on agar slants, into yeast extract peptone medium containing 5% mannitol. This preparation was allowed to grow on a reciprocal laboratory shaker operating at two hundred 1 1/2-inch strokes per minute. At the end of four weeks the contents of the flask were poured into a sterilized steel Waring Blendor cup and blended into a fine even suspension. Five milliliters of the suspension were inoculated into each flask of test medium after which the flasks were placed on the mechanical shaker. After a 15 day growth period, (see Fig. 1) the flasks were harvested by filtering the growth through tared sintered glass crucibles and thoroughly washed with distilled water to remove all adhering constituents of the medium. The growth was dried as much as possible using a Buchner flask and vacuum and then placed in a laboratory homophizer to remove com

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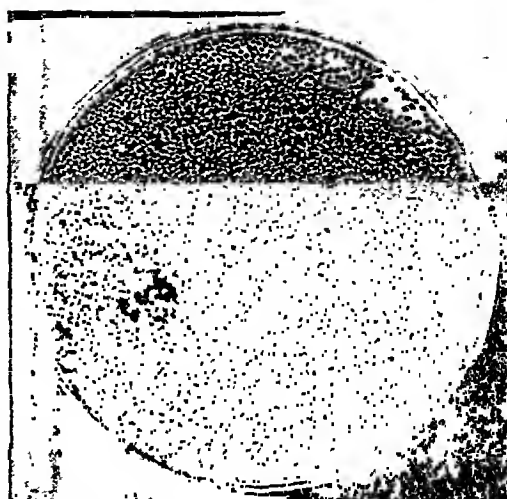


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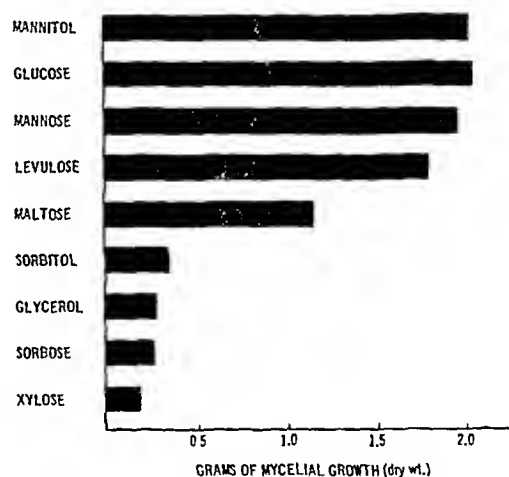


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It was noted that the mannitol-grown mycelium produced large amounts of fat-like material while the tissues grown on other carbohydrates produced only small amounts. The production of large amounts of fats and sterols is a condition also found in natural ergot.

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produced any detectable amounts of alkaloids, mannitol could be considered the most desirable carbon source since mycelial tissues grown on it produced by comparison more characteristics resembling the natural sclerotia than did the mycelia grown on the other carbon compounds.

REFERENCES

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The Oxidative Metabolism of Certain Krebs Cycle Acids in Tissue Cultures of *Claviceps litoralis**

By W. T. GLOOR, Jr.† and H. W. YOUNGKEN, Jr.‡

An oxidative metabolism of acetic, pyruvic, succinic, fumaric and malic acids is demonstrated in mycelial cultures of *Claviceps litoralis* Kawatani. Carbon-14 from carboxyl-labeled sodium acetate added to cultures was found to be incorporated in all but one of these acids (succinate). However no detectable amounts of tricarboxylic acids could be isolated from the mycelial tissues. It is concluded that a complete Krebs cycle probably does not exist in the fungus when it is grown under the synthetic culture conditions described.

SINCE the elucidation by Krebs (1) of the tricarboxylic acid cycle in mammalian tissues, many attempts have been made to show the presence of this metabolic cycle as an oxidative pathway in plants and microorganisms. Re-

cently Millerd *et al.* (2) have shown that the complete Krebs cycle exists in mung bean seedling mitochondria. This is the first work to demonstrate the presence of the complete cycle in higher plants. More recently, Krebs *et al.* (3), Krampitz *et al.* (4-6) and others (7-9) have reported conclusive evidence for the existence of the entire cycle in microorganisms.

In view of the importance of the TCA cycle in the oxidative metabolism of carbohydrates in various organisms, it was thought that if such a pathway could be demonstrated in a *Claviceps spp.*, this might serve as a starting point for further attempts to elucidate a biosynthetic pathway of alkaloid formation in *Claviceps*. In the studies cited above, manometric data have been an important part of the evidence presented. However, preliminary manometric studies proved quite disappointing because of the high rate of endogenous respiration of the *C. litoralis* tissues. When attempts to overcome this problem proved unsatisfactory, it became necessary to use some other method in an attempt to demonstrate the

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presence of a TCA cycle. This report describes the culturing of *Claviceps litoralis*, Kawatani, on carbon-14 carboxyl-labeled sodium acetate and the subsequent isolation of carbon-14 labeled TCA cycle components.

EXPERIMENTAL

Culture Method—Inoculation cultures of *Claviceps litoralis* mycelial tissues were made by inoculating liquid medium by means of an inoculating needle, from a stock culture agar slant. The liquid medium used in this study consisted of 5% mannitol, 1.25% yeast extract, 0.25% KH_2PO_4 , 0.125% peptone and 0.125% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. These cultures, which consisted of 100 ml. of inoculated medium in a 500-ml. Erlenmeyer flask, were continuously shaken on a reciprocal shaker operating at 200 strokes per minute. After four weeks the cultures were removed and the contents of the flask whipped into a fine suspension in a sterile Waring Blendor eup. This suspension was used to inoculate other flasks of yeast-extract-peptone medium to produce the growth used in this study. Five milliliters of the suspension were inoculated into each flask of 100 ml. of medium. These flasks were then allowed to grow on the reciprocal shaker for 15 days after which the growth was filtered and washed clean of adhering medium constituents with distilled water. Under these conditions the growth consisted mainly of small, roughly-spherical pellets approximately 1-3 mm. in diameter.

The pellets of fungal growth material were then handled in two ways. One group, approximately a third of the material, was not treated in any way and will be referred to as "freshly harvested material" or "unstarved material." The organic acids were extracted and isolated from this material as described below.

The remainder of the mycelial pellets, usually about 200 grams (wet weight), was placed in 1 liter of 0.1 *M* phosphate buffer solution (pH 6.8). This mixture was aerated vigorously in a covered 4-liter beaker for 3 days. During the 3-day period the buffer solution was replaced with fresh buffer solution at 4- to 12-hour intervals. At first, the aerating solution was checked several times every day for possible bacterial contamination; but this practice was discontinued later when no evidence of contamination appeared. The pellets treated in the above fashion will be referred to as "starved" mycelium.

The "starved" material was divided into two groups. One part of the mycelial material was given no additional treatment but extracted for organic acids as described below. The second portion of the "starved" material was treated with carbon-14 carboxyl-labeled sodium acetate. To carry out this treatment an arrangement of apparatus was used which was similar to that employed by Djao (10) (Fig. 1). A 500-ml. Erlenmeyer flask was fitted with an inlet tube connected to a bubble trap containing barium hydroxide solution and an outlet tube which led to a series of two bubble traps containing standardized barium hydroxide solution. The second bubble trap was connected to a vacuum flask. Into the Erlenmeyer flask were placed 30 Gm. (wet weight) of "starved" mycelium pellets,

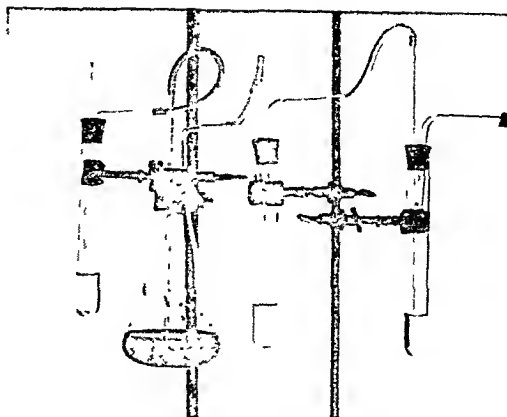


Fig. 1.—Apparatus for collection of carbon-14 labeled carbon dioxide released by *Claviceps litoralis* from carboxyl labeled sodium acetate

116 ml. of 0.1 *M* phosphate buffer solution and 4 ml. of labeled sodium acetate solution, 0.3 *M* (described below). When a vacuum was applied to this system, carbon dioxide-free air was admitted through the inlet tube into the flask, agitating the mixture and flushing the carbon dioxide from the flask into the barium hydroxide solution in the bubble traps. The barium carbonate precipitate was used for the measurement of radioactivity. The mycelium was treated in this manner and the carbon dioxide collected over a period of 2 hours. Then the mycelium was removed from the solution by filtering, washed free of acetate and the organic acids extracted. After the individual acids had been separated and identified, they were oxidized separately as described later and their radioactivity content determined.

Tracer Material and Technique—Carbon-14 carboxyl-labeled sodium acetate having a labeled activity of 2 μe per millimole was diluted with sufficient nonradioactive sodium acetate and dissolved in distilled water to give a 0.3 *M* acetate solution. When 1 ml. of this solution was diluted to 30 ml., the final concentration of acetate was 0.01 *M* and the activity was approximately 25,000 counts per minute. Specific activity (counts/minute/mg. of carbon) of this solution was 3.35×10^4 .

A convenient method of oxidizing samples in preparation of isotope assays is by the wet oxidation method (11) which was employed in this study. The carbon dioxide is absorbed in a 0.25 *N* barium hydroxide containing 2% barium chloride. The total amount of barium carbonate is determined by titrating the excess barium hydroxide with 0.12 *N* hydrochloric acid using phenolphthalein as the indicator and calculating the amount of carbonate from the difference between the blank and the sample titrated. The barium carbonate precipitate was then collected and washed three times with 25 ml. portions of 95% ethanol. Several aliquots of each carbonate precipitate were homogenized in ethanol and plated on copper disks. Radioactivity measurements were made with a windowless flow counter (Tracerlab Windowless Flow Counter, Model SC-16) and an automatic scaling unit (Nuclear Scaling Unit, Model 163). These measurements were then corrected for background count (usually

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presence of a TCA cycle. This report describes the culturing of *Claviceps litoralis*, Kawatani, on carbon-14 carboxyl-labeled sodium acetate and the subsequent isolation of carbon-14 labeled TCA cycle components.

EXPERIMENTAL

Culture Method—Inoculation cultures of *Claviceps litoralis* mycelial tissues were made by inoculating liquid medium by means of an inoculating needle, from a stock culture agar slant. The liquid medium used in this study consisted of 5% mannitol, 1.25% yeast extract, 0.25% KH_2PO_4 , 0.125% peptone and 0.125% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. These cultures, which consisted of 100 ml. of inoculated medium in a 500-ml. Erlenmeyer flask, were continuously shaken on a reciprocal shaker operating at 200 strokes per minute. After four weeks the cultures were removed and the contents of the flask whipped into a fine suspension in a sterile Waring Blender cup. This suspension was used to inoculate other flasks of yeast-extract-peptone medium to produce the growth used in this study. Five milliliters of the suspension were inoculated into each flask of 100 ml. of medium. These flasks were then allowed to grow on the reciprocal shaker for 15 days after which the growth was filtered and washed clean of adhering medium constituents with distilled water. Under these conditions the growth consisted mainly of small, roughly-spherical pellets approximately 1-3 mm. in diameter.

The pellets of fungal growth material were then handled in two ways. One group, approximately a third of the material, was not treated in any way and will be referred to as "freshly harvested material" or "unstarved material." The organic acids were extracted and isolated from this material as described below.

The remainder of the mycelial pellets, usually about 200 grams (wet weight), was placed in 1 liter of 0.1 *M* phosphate buffer solution (pH 6.8). This mixture was aerated vigorously in a covered 4-liter beaker for 3 days. During the 3-day period the buffer solution was replaced with fresh buffer solution at 4- to 12-hour intervals. At first, the aerating solution was checked several times every day for possible bacterial contamination; but this practice was discontinued later when no evidence of contamination appeared. The pellets treated in the above fashion will be referred to as "starved" mycelium.

The "starved" material was divided into two groups. One part of the mycelial material was given no additional treatment but extracted for organic acids as described below. The second portion of the "starved" material was treated with carbon-14 carboxyl-labeled sodium acetate. To carry out this treatment an arrangement of apparatus was used which was similar to that employed by Djao (10) (Fig. 1). A 500-ml. Erlenmeyer flask was fitted with an inlet tube connected to a bubble trap containing barium hydroxide solution and an outlet tube which led to a series of two bubble traps containing standardized barium hydroxide solution. The second bubble trap was connected to a vacuum flask. Into the Erlenmeyer flask were placed 30 Gm. (wet weight) of "starved" mycelium pellets,

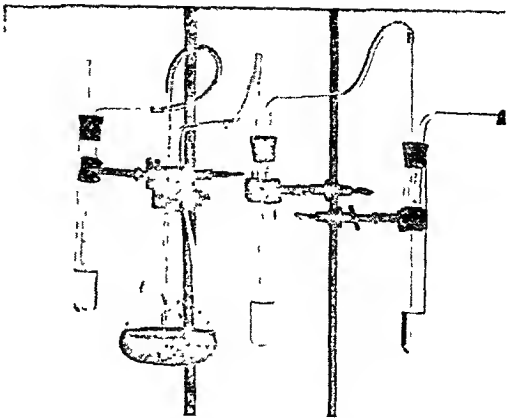


Fig. 1.—Apparatus for collection of carbon-14 labeled carbon dioxide released by *Claviceps litoralis* from carboxyl labeled sodium acetate

116 ml. of 0.1 *M* phosphate buffer solution and 4 ml. of labeled sodium acetate solution, 0.3 *M* (described below). When a vacuum was applied to this system, carbon dioxide-free air was admitted through the inlet tube into the flask, agitating the mixture and flushing the carbon dioxide from the flask into the barium hydroxide solution in the bubble traps. The barium carbonate precipitate was used for the measurement of radioactivity. The mycelium was treated in this manner and the carbon dioxide collected over a period of 2 hours. Then the mycelium was removed from the solution by filtering, washed free of acetate and the organic acids extracted. After the individual acids had been separated and identified, they were oxidized separately as described later and their radioactivity content determined.

Tracer Material and Technique—Carbon-14 carboxyl-labeled sodium acetate having a labeled activity of 2 μc per millimole was diluted with sufficient nonradioactive sodium acetate and dissolved in distilled water to give a 0.3 *M* acetate solution. When 1 ml. of this solution was diluted to 30 ml., the final concentration of acetate was 0.01 *M* and the activity was approximately 25,000 counts per minute. Specific activity(counts/minute/mg. of carbon) of this solution was 3.35×10^4 .

A convenient method of oxidizing samples in preparation of isotope assays is by the wet oxidation method (11) which was employed in this study. The carbon dioxide is absorbed in a 0.25 *N* barium hydroxide containing 2% barium chloride. The total amount of barium carbonate is determined by titrating the excess barium hydroxide with 0.12 *N* hydrochloric acid using phenolphthalein as the indicator and calculating the amount of carbonate from the difference between the blank and the sample titrated. The barium carbonate precipitate was then collected and washed three times with 25 ml. portions of 95% ethanol. Several aliquots of each carbonate precipitate were homogenized in ethanol and plated on copper disks. Radioactivity measurements were made with a windowless flow counter (Tracerlab Windowless Flow Counter, Model SC-16) and an automatic scaling unit (Nuclear Scaling Unit, Model 163). These measurements were then corrected for background count (usually

20 \pm 2 counts/minute) and self absorption (see Fig 2) and calculated as specific activity (counts/minute/mg of carbon)

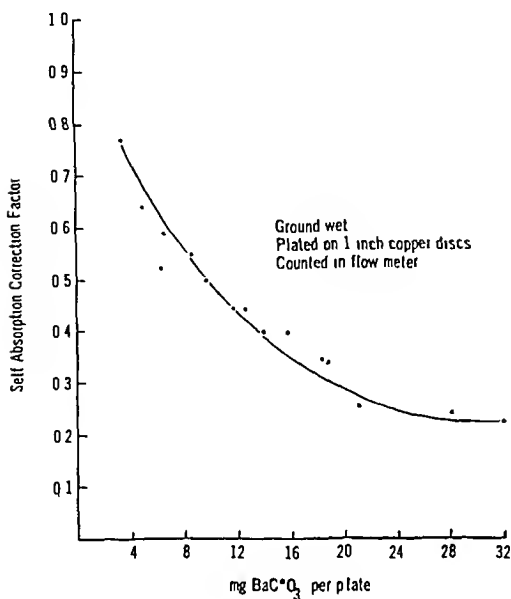


Fig 2—Self absorption correction curve for BaC*O₃

Samples of mycelium material were dried completely in a lyophilizer prior to oxidation. Samples of acetate or other radioactive liquids were neutralized with sodium hydroxide solution and evaporated to dryness under reduced pressure before oxidation. If the activity of a sample was too high, a smaller aliquot of the material was diluted with nonradioactive glucose and oxidized again in order to keep the counting rate between 100 and 2000 counts per minute.

Extraction and Isolation of Organic Acids—The organic acids were extracted by boiling 30 Gm (wet weight) of mycelium pellets in 30 ml of 0.1 N sodium hydroxide and filtering. The residue was re-treated in the same manner three times. The aqueous extracts were combined and neutralized, if necessary, with sodium hydroxide solution and evaporated to dryness. The dried extract was then taken up with 2.5 ml of 0.2 N H₂SO₄ and chromatographed using the method of Eaton and Klein (12). At least four samples of each of the three groups of mycelial pellets described above were extracted and chromatographed. Each fraction was titrated with 0.01 N NaOH and the amount of acid in each fraction calculated. This information was plotted and the amount of each individual acid determined. The organic acids isolated from the acetate-treated, "starved" material were oxidized, their radioactivity counted, and specific activity calculated. The results are shown in Table I.

DISCUSSION AND CONCLUSIONS

From the results obtained above it appeared that an oxidative cycle involving some of the compo-

TABLE I—COMPARISON OF ORGANIC ACID CONCENTRATION IN FRESH PELLETS, STARVED PELLETS AND ACETATE-TREATED PELLETS OF *CLAVICEPS LITORALIS*

	Mg Acid Isolated from Fresh Pellets	Mg Acid Isolated from Starved Pellets	Mg Acid Isolated Acetate treated Starved Pellets ^a	Specific Activity ^b
Acetate	2.56	0.94	1.57	262
Pyruvate		2.75	7.28	335
Succinate		1.57		
Fumarate	0.30	1.00	3.74	1307
Malate	0.32	1.43	3.28	2658
Total Acids	3.18	7.69	15.87	

^a Carbon 14 carboxyl labeled sodium acetate

^b Specific activity = counts per minute per mg carbon

nents of the TCA cycle was in operation. A significant amount of acetate was isolated from the fresh "unstarved" material along with small amounts of fumarate and malate. The entire amount of acetate isolated should not be considered as coming from such an oxidative cycle, for, when a general extraction method is used, all acetate, whether "active" acetate or acetate from a metabolic "acetate pool," will be extracted.

The "starved" material showed approximately a 100 per cent increase in total acids isolated compared to the amount found in the fresh material, indicating perhaps that the conditions of aeration and starvation enhanced the oxidative processes and forced the organism to utilize reserve materials. The "starved," acetate treated material showed a 200 per cent increase in total acids. In both latter cases acetate was found in the least amount while pyruvate appeared in the greatest quantity. Fumarate and malate appeared in approximately the same amounts in each separate group of material. It is reasonable to believe that a portion of the carbohydrate would be oxidized through the cycle. With the cycle operating at a higher rate than in the fresh material, it could oxidize the labeled acetate at a more efficient pace.

In all cases no citrate or isocitrate could be isolated. This would indicate that the entire TCA cycle as elucidated by Krebs was not functioning; however, these acids may have been present in minute amounts too small to be detected or they may have been absent. The absence of citrate and isocitrate could also be explained on the basis of the work by Ochoa *et al* (13, 14). These workers reported the finding of an enzyme present in microorganisms which not only decarboxylated l-malic acid, but also decarboxylated oxalacetic acid. The result of these decarboxylations was the formation of pyruvate which, in turn, was converted to lactic acid by lactic dehydrogenase. However, since lactic acid could not be detected in *C. litoralis*, the possibility exists that lactic dehydrogenase was lacking or might have been inactive in the fungus allowing an accumulation of pyruvate to occur. Considering the above two points, it would seem reasonable to believe that the formation of citrate would be prevented to a great extent and such a situation would also explain the accumulation of pyruvate.

Although the TCA cycle could be prevented from operating in its entirety by such an enzyme, the cycle would exist in part if, through the Thunberg-Wieland reaction, the acetate were incorporated into the cycle in the form of succinate. Succinate was isolated from the "starved," untreated material, but the failure to isolate succinate from the "starved," acetate-treated mycelial pellets weakens such a hypothesis unless the presence of a highly active form of succinic dehydrogenase could be shown to exist in *C. litoralis*. No work along this line was attempted. From succinate the carbon could follow the pathway through fumarate and malate to pyruvate, and this acid could then be decarboxylated to form acetate.

From the results obtained it appears that the entire TCA cycle does not function in *C. litoralis* when it is grown under artificial conditions used in this study, but a partial cycle does exist as an oxidative pathway in this fungus. However, considering the low specific activity of the isolated, labeled acids, it is possible that some other oxidative pathway plays a more important role in the oxidative

metabolism of *C. litoralis* than does any organic acid cycle which may resemble the complete TCA cycle

REFERENCES

- (1) Krebs, H. A., *Advances in Enzymol.*, **3**, 191(1943)
- (2) Miller, A., Bonner, J., Axelrod, B., and Bandurski, R. S., *Proc. Nat. Acad. Sci., U. S.*, **37**, 855(1951)
- (3) Krebs, H. A., Gurin, S., and Eggleston, L. V., *Biochem. J.*, **51**, 614(1952).
- (4) Saz, H. J., and Krampitz, L. O., *J. Bacteriol.*, **67**, 409(1954)
- (5) Swim, H. E., and Krampitz, L. O., *ibid.*, **67**, 419, 426(1954)
- (6) Saz, H. J., and Krampitz, L. O., *ibid.*, **69**, 288(1955)
- (7) Lewis, K. F., and Weinhouse, S., *J. Am. Chem. Soc.*, **73**, 2500, 2906(1951)
- (8) Englesberg, E., and Levy, J. B., *J. Bacteriol.*, **69**, 418(1955)
- (9) Ochoa, S., *Advances in Enzymol.*, **15**, 183(1954)
- (10) Djaio, E. H., and Youngken, H. W., Jr., *This Journal*, **43**, 425(1954)
- (11) Calvin, M., Heidelberger, C., Reid, J. C., Talbert, B. M., and Yankwich, P. E., "Isotopic Carbon," John Wiley & Sons, Inc., New York, 1949, pp. 92-94
- (12) Eaton, N. R., and Klein, H. P., *J. Bacteriol.* To be Published
- (13) Ochoa, S., *Advances in Enzymol.*, **15**, 183(1954)
- (14) Korles, S., Del Campillo, A., and Ochoa, S., *J. Biol. Chem.*, **187**, 891(1950).

A Comparative Study of Surfactant Influence on the Release of Ions from an Emulsified Ointment Base*

By JOHN F. STARK†, JOHN E. CHRISTIAN, and H. GEORGE DeKAY

An apparatus has been designed and an *in vitro* method employing radioisotopes has been developed to study medicament release from pharmaceutical semisolids. Radio-labeled mercuric and iodide ions have been incorporated into hydrophilic bases emulsified with 1, 3, and 5 per cent concentrations of anionic, cationic, and nonionic surface active agents and the ability of the bases to release the contained ions has been measured

fraction of the hydrophilic bases

The release patterns of a modified U. S. P. XV hydrophilic ointment base (1), emulsified with various concentrations of ionic and nonionic surface active agents, have been measured over predetermined time intervals. The effect of surfactant classification, concentration and electrostatic influence on the ability of the emulsified vehicles to release the labeled ions has been studied

EXPERIMENTAL

Design of the Apparatus.—The apparatus (Fig 1) supported a section of 3/4 inch standard stretch seamless cellulose tubing,¹ securely attached to the apparatus in such a manner as to support a slight positive head of pressure and thus withstand the contact of the circulating liquid without leaking. The cellulose enabled more reproducibility of results than is usually possible with a living excised membrane.

The counting chamber was designed so as to allow fluid flow around the counter housing without actually wetting and contaminating the contained Geiger tube. An RIDL Model 200 Scaler² in conjunction with a bismuth cathode side-window

THE SUBSERVIENT of the newer hydrophilic bases must of necessity be a measure of their ability to release a contained medicinal agent. The hydrophilic character of the emulsified semisolid, the surfactant classification, concentration and particle size, and the solubility and pH of the incorporated medicament appear, among other factors, to influence the release pattern.

An *in vitro* method, simulating the actual conditions under which a medicated ointment is frequently used, was developed to study medicament release. Because of the ease and extreme sensitivity of detection, radioisotope labeled compounds were employed to represent the medicinal

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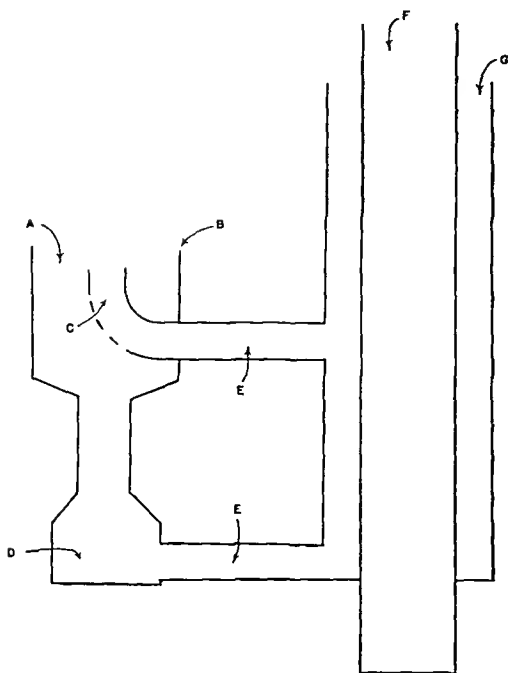


Fig 1—Diagram of the release apparatus. A, Membrane Station, B, Ground Glass Surface, C, Perforated Extension (to prevent an ionic concentration gradient below the membrane), D, Stirring Compartment, E, Connecting Arms, F, Counter Housing, G, Counting Chamber

counter,³ geometrically seated in the counter housing, was used to determine the radioactivity. Shielding, in the form of lead shot contained in a copper housing designed to fit across the connecting arms, protected the counter from external sources of ionizing radiation.

A physiological solution containing the chlorides of sodium, potassium, calcium, and magnesium (2) was used as the circulating liquid. A rheostat controlled magnetic bar, enclosed in the stirring station, provided the necessary circulation.

A constant temperature bath of 2 liter capacity was constructed of sheet copper to accommodate the release apparatus. All experimental work was conducted at $37^{\circ} \pm 1^{\circ}$.

The bases containing the labeled ions were placed in a lucite holder and carefully weighed for application to the membrane. The holder was cut from 7-mm stock, 50 x 65 mm and a hole 32 mm in diameter was bored in the center of the section. The lucite was backed with aluminum foil, forming a cup to support bases of uniform thickness, known weight, and total activity.

Base Preparation.—The U S P XV hydrophilic ointment base was modified by using selected ionic and nonionic surfactants in 1, 3, and 5% concentrations as emulsifying agents. A constant aqueous phase was maintained by adjusting the amounts of white petrolatum and stearyl alcohol to accommodate the varying surfactant levels. A portion of the water was omitted in the formulation and sub-

sequently replaced with dilutions of the radioactive solutions. With other than these changes, the U S P method of formulation was followed.

The general formulas of the bases used are shown in Table I.

TABLE I

Ingredients	Quantities Gm		
	Surfactant 1	Concn 3	Per Cent 5
Methylparaben	0.25	0.25	0.25
Propylparaben	0.15	0.15	0.15
Stearyl alcohol	27.0	26.0	25.0
White petrolatum	27.0	26.0	25.0
Emulsifier	1.0	3.0	5.0
Propylene glycol	12.0	12.0	12.0
Purified water	23.0	23.0	23.0
Labeled dilution ^a	10.0	10.0	10.0
To make about	100.0	100.0	100.0

^a Refers to either NaI¹³¹ or Hg²⁰³(NO₃)₂ dilutions contain mg, 50 mg/ml of carrier.

Sodium radio-iodide (I¹³¹) and mercury (Hg²⁰³) nitrate,⁴ containing a labeled anion and cation respectively, were employed to simulate the medication fractions of the bases. These isotopes have convenient half-lives and emit characteristic gamma radiations of nearly the same energy. Dilutions containing 50 mg/ml of carrier were prepared so that the volume incorporated imparted 1,500 to 5,000 counts per minute⁵ as determined by the procedure described below.

A portion of the base containing the labeled medicament was placed in a 2 dram, collapsible, tin tube for convenience in filling the lucite holder and weighing of the standardization sample.

Base Standardization.—An accurately weighed representative sample of radioactive ointment of approximately 0.5 Gm was dispensed from the collapsible tube into a 100 ml volumetric flask. The base was dissolved in a warm mixture of equal parts of ethyl alcohol and chloroform, the solution cooled, and brought to volume with the solvent mixture.

The 100 ml of solution was added to a standardization apparatus modeled after and identical to the counting section of the release apparatus and the radioactivity determined. The activity was expressed in terms of CPM per unit weight of base and was used to calculate the total activity of the base applied to the membrane in the release study.

The standardization of the base under conditions identical to those in the determination of the release activity enabled expression of the final results in terms of per cent of the total medicament applied to the membrane.

Medicament Release Studies.—The magnetic bar was placed into the stirring compartment and the membrane attached to the release apparatus. The physiological solution was added and the air pocket beneath the membrane was removed by displacement. The apparatus was stationed in the 37° bath, the shielding was placed in position, and the counter was inserted into the housing. Rheostat controlled stirring was commenced and an additional physiological solution was added up to a

⁴ Obtained from Oak Ridge National Laboratory, Oak Ridge, Tenn. Operated for the United States Atomic Energy Commission by Union Carbide Nuclear Company.

⁵ Counts per minute hereinafter referred to as CPM.

³ Nuclear Chicago, Chicago, Ill.

predetermined and constant height on the counter housing. This insured that the liquid level with regard to the pre-positioned counter was identical to that used in the standardization procedure and subsequent determinations.

Background activity determinations were made in each instance with the lucite holder (foil side down) in position on the membrane; thus, any small fraction of the ionizing radiation which penetrated the shielding was recorded as part of the background. The lucite holder was then inverted and the foil backing of the holder was rubbed gently to promote uniform contact between the base and the membrane.

A stopwatch was started immediately after applying the base to the membrane and after four minutes had elapsed, the radioactivity of the physiological solution was determined over a period of two minutes. The corrected activity, expressed in CPM, was divided by the total activity of the base applied to the membrane, and multiplied by 100. This relative value, expressed as per cent, was used as a measure of medicament release at the five-minute interval. Similar activity measurements indicated the release at the end of 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, and 170 minutes.

The experimental data for iodide and mercuric ion are presented in Table II. Although the release measurements were conducted at sixteen different time intervals, only values indicative of the release at the end of two hours are reported for conciseness.

DISCUSSION

A survey of the data revealed that the ability of the hydrophilic bases to release the ions under study followed a characteristic pattern, especially with regard to surfactant concentration and type.

Maximum release of both the iodide ion and mercuric ion was evidenced from bases emulsified with 1% surfactant concentrations. The bases emulsified with 3% concentrations exhibited a decreased ability to release the incorporated ions. At the 5% level the release was generally further retarded. In an earlier publication Barker, *et al.* (3), reported that iodide was released to a greater extent from hydrophilic bases emulsified with low rather than higher levels of nonionic surfactants, as measured by a modified agar plate method. The results obtained by the method reported here are in agreement with Barker's findings. Maximum release within each group, ionic or nonionic, was generally noted at the lowest concentration level employed, namely 1%.

The ionic or nonionic nature of the agents utilized to emulsify the vehicles substantially influenced the release. The nonionic group of surfactant promoted the release of both the iodide and mercuric ions to a greater extent than did the anionic or cationic group. In the case of iodide ion, the anionic surfactants provided for greater release than the cationic; whereas, in the case of the mercuric ion there was no significant difference between the effects of these two types.

TABLE II.—THE RELEASE OF IODIDE AND MERCURIC IONS^a FROM HYDROPHILIC OINTMENT BASES^b EMULSIFIED WITH 1, 3, AND 5 PER CENT CONCENTRATIONS OF IONIC AND NONIONIC SURFACE ACTIVE AGENTS

Surfactant Listing ^c	Iodide Ion Released ^d , Concn of Surfactant, %			Mercuric Ion Released ^d , Concn of Surfactant, %		
	1	3	5	1	3	5
Aerosol® OT	18.5	13.2	9.8	8.1	4.6	3.5
Antarate K	14.5	9.1	10.9	5.7	2.3	1.7
Blendene	20.4	14.7	11.3	5.0	3.9	3.2
Emulgade F	18.1	16.2	16.6	7.7	5.6	3.8
Sodium lauryl sulf.	17.4	16.3	14.8	6.3	5.9	6.1
Soluble base 11	15.6	12.2	9.8	5.9	2.1	2.5
BTC 100%	7.9	7.8	6.7	3.2	1.1	1.0
Emcol E-607S	12.0	8.5	6.4	6.7	2.4	2.0
Ethomeen C/15	16.7	19.4	17.3	6.7	2.6	2.8
Ethyl cetab	11.4	8.0	8.0	5.7	1.6	1.6
Katapol PN-430	14.7	8.6	7.0	4.8	2.4	2.1
Triethanolamine	11.3	10.4	8.9	6.2	5.0	2.9
Brij® 35	21.8	15.4	16.2	10.3	5.1	3.7
Emulsifier L-32	20.0	4.7	4.6	6.2	4.0	2.4
Ethomid C/15	13.7	12.5	10.9	7.5	4.7	3.3
G-2000C	21.0	11.9	9.9	11.5	7.0	4.3
G-2152	22.9	19.1	16.4	10.7	7.1	5.9
G-7596J	23.9	17.2	18.3	11.4	6.5	5.6
Myrj® 52	21.1	17.5	17.2	9.9	8.9	6.1
Neutronyl 834	20.9	7.7	4.4	7.5	3.0	3.7
Nonisol® 100	20.2	16.9	15.8	10.6	6.0	5.5
Promulgen	22.7	17.9	14.8	13.0	7.9	6.4
Tween® 40	26.1	17.6	19.0	12.4	6.6	4.9
Tween 80	26.3	20.3	20.4	8.8	6.4	2.5

^a The iodine and mercury were in the chemical forms NaI and Hg(NO₃)₂ respectively.

^b The hydrophilic bases were modifications of the U. S. P. XV hydrophilic ointment. Ten per cent water was omitted in formulating the bases and subsequently replaced with the labeled dilutions. The aqueous phase of each base was constant and the increased amounts of emulsifier were compensated for by equally reducing the amounts of stearyl alcohol and white petrolatum.

^c Surfactants 1-6 are classified anionic; 7-12 are classified cationic; and 13-24 are classified nonionic. J. W. McCutcheon's "Synthetic Detergents and Emulsifiers—Up To Date," III, 1955, may be consulted for suppliers of the surface-active agents employed in this study.

^d The values reported represent the medicament released at the end of 120 minutes. A study of the variation that could be expected between individual determinations was conducted and percentage errors of 2.3 to 4.1 were found, well within the expected deviation.

CONCLUSION

It can be generally stated that maximum release of a medicinal agent may be expected from a base emulsified with a nonionic surfactant in as low a concentration as is feasible to impart the required emulsion character and stability; however, the release of a given medicament is dependent upon many factors and each formula-

tion must be considered individually for medication release characteristics.

REFERENCES

- (1) "Pharmacopeia of the United States of America," 15th rev., 1955, p. 469. Mack Publishing Co., Easton, Pa.
- (2) Fox, C. L., Winfield, J. M., Slobody, L. B., Swindler, C. M., and Lattimer, J. K., *J. Am. Med. Assoc.*, 148, 827 (1952).
- (3) Barker, D. Y., Christian, J. E., and DeKay, H. G., *THIS JOURNAL*, 45, 601 (1956).

The Paper Chromatography and Electrophoresis of Selected Therapeutic Agents I.*

The Paper Chromatography of Antihistaminic Agents

By BETLEIGH C. COX, HAROLD C. HEIM, and CHARLES F. POE

The paper chromatography of some antihistamines has been performed with six solvent systems. Pretreatment of the paper with monosodium citrate resulted in consistently lower and more widely varying R_f values, in all of the solvent systems used. A greater degree of resolution was obtained. The solvent systems that provided the most successful resolution of a mixture of the antihistamines were: (a) isoamyl alcohol-water-citric acid, 50:50:3 Gm.; (b) *n*-butanol-water-amyl acetate, 10:1:4 v/v; and (c) isoamyl alcohol-water-ethyl acetate, 10:1:3 v/v.

THERE ARE NUMEROUS antihistaminic compounds in use today, and many exhibit similarities of chemical structure. The problem of identification and resolution of these substances has become complex. The antihistamines have been analyzed by means of their melting points, spectrophotometric patterns (1, 2), polarographic curves (3), qualitative reactions (4, 5), and ion-exchange resin behavior (6).

Paper chromatography offers a method of identification and recovery of minute amounts of these compounds. The resolution of a mixture of the antihistamines is made possible by the use of this technique. This paper describes the paper chromatographic behavior of a representative group of the antihistamines.

EXPERIMENTAL

Apparatus.—The chromatographic chamber was a Research Equipment Corp. Chromatocab, Model A 300.

Materials.—All solvents and chemicals were C. P. or Analytical Reagent grade.

The following compounds were studied: Diphenhydramine hydrochloride (Benadryl®), thonzyl-

mine hydrochloride (Neo-hetramine®), doxylamine succinate (Decapryn®), tripeleennamine hydrochloride (Pyribenzamine®), chlorcyclizine hydrochloride (Di-Paralene®), pyrilamine maleate (Neo-Antergan®), pyriethazine hydrochloride (Pyrrol-zote®), antazoline hydrochloride (Antistine®), methapyrilene hydrochloride (Thenylene®), pheniramine maleate (Trimeton®), chlorpheniramine maleate (Chlor-Trimeton®) and phenindamine tartrate (Thephorin®).

Solvent Systems.—(a) *n*-Butanol-water-citric acid, 50:50:3 Gm.; (b) isoamyl alcohol-water-citric acid, 50:50:3; (c) *n*-butanol-water-ethyl acetate, 10:2:1 v/v; (d) *n*-butanol-water-ethyl acetate, 10:1:2 v/v; (e) isoamyl alcohol-water-ethyl acetate, 10:1:3 v/v; (f) *n*-butanol-water-amyl acetate, 10:1:4 v/v.

The mobile phase was the organic layer, in all instances. In all experiments involving *n*-butanol, fresh solvent was necessary for each resolution to achieve reproducible R_f values.

Paper.—Whatman No. 1 chromatography paper, in strips 34.0 cm. long and 1.3 cm. wide, was used. Pretreated strips were used in each solvent system. Pretreatment consisted of thoroughly wetting the paper in 5 per cent aqueous monosodium citrate, blotting the excess solution and drying at 70° for 20 minutes.

Experimental Procedure.—Descending chromatography was used. A solution of approximately 25 mcg. of antihistamine in 95% ethanol was applied to each strip. After drying, the strips were placed in the Chromatocab for an equilibration period of

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nine hours. A constant volume of 150 ml. of developing solvent was added to the trough in all experiments. Seventy-five ml. of the aqueous phase were placed in the bottom of the chamber. Development was performed at $23^{\circ} \pm 0.5^{\circ}$. The strips were air dried and sprayed with modified Dragendorff reagent (7) for detection of the compounds.

RESULTS AND DISCUSSION

The R_f values of the antihistamines, as determined in the various solvent systems, are listed in Table I. Although 18 determinations were made with each antihistamine, only the most characteristic ones are included in the table. Also, the results with untreated paper are omitted. Some experiments were performed in which the ascending method was used.

high R_f values, and poor resolution. Lactic acid as the third component produced no improvement. The solvent system *n*-butanol-water-citric acid, 50:50:1 Gm. gave lower R_f values, but unsatisfactory resolution, upon untreated strips. However, pretreated strips produced considerably improved resolution of the compounds. Increasing the citric acid content to 3 Gm. further improved the resolution. When the citric acid content was increased to 5 Gm., the resolution was decreased because there were a group of R_f values in a high range, and a group in a low range. Thus, it appears that optimum resolution of these compounds is attained with the solvent system *n*-butanol-water-citric acid 50:50:3.

When isoamyl alcohol was used as the solvent, the R_f values were consistently lower than when *n*-butanol was used. Some compounds, such as chlorcyclizine hydrochloride, pyrazinazine hydrochloride, and phenindamine tartrate, exhibited high

TABLE I.— R_f VALUES OF ANTIHISTAMINES

Compound	Solvent Systems ^a					
	1	2	3	4	5	6
Diphenhydramine hydrochloride	0.87	0.75	0.94	0.68	0.72	0.65
Thonzylamine hydrochloride	0.79	0.56	0.88	0.56	0.45	0.49
Doxylamine succinate	0.39	0.16	0.59	0.38	0.20	0.28
Tripelennamine hydrochloride	0.49	0.23	0.70	0.42	0.28	0.31
Chlorcyclizine hydrochloride	0.97	0.79	0.97	0.80	0.77	0.69
Pyrimilamine maleate	0.48	0.31	0.68	0.39	0.26	0.33
Pyrazinazine hydrochloride	0.90	0.88	0.96	0.79	0.74	0.76
Antazoline hydrochloride	0.88	0.84	0.95	0.78	0.67	0.63
Metapyrilene hydrochloride	0.50	0.19	0.73	0.44	0.29	0.35
Pheniramine maleate	0.44	0.25	0.61	0.40	0.23	0.24
Chlorpheniramine maleate	0.78	0.49	0.86	0.49	0.35	0.43
Phenindamine tartrate	0.89	0.81	0.93	0.73	0.70	0.72

^a 1. *n*-Butanol-water-citric acid, 50:50:3 Gm., with pretreated paper. 2. Isoamyl alcohol-water-citric acid, 50:50:3 Gm., with pretreated paper. 3. *n*-Butanol-water-ethyl acetate, 10:2:1 v/v, with pretreated paper. 4. *n*-Butanol-water-ethyl acetate, 10:1:2 v/v, with pretreated paper. 5. Isoamyl alcohol-water-ethyl acetate, 10:1:3 v/v, with pretreated paper. 6. *n*-Butanol-water-amyl acetate, 10:1:4 v/v, with pretreated paper.

With the apparatus used in this study, the R_f values were found to be essentially the same as those obtained with the descending method.

The use of a large chromatographic chamber necessitated an adequate equilibration period and a strict control of quantities of mobile and stationary solvent phases. The development period of nine hours produced a solvent flow of 20.0 to 22.0 cm., and increased accuracy. R_f values were slightly erratic if the solvent flow was permitted to reach a point two or three cm. from the end of the strip.

Pretreatment of the paper with monosodium citrate produced a significant improvement in the chromatography of these compounds. The spots were more discrete and more readily visible. The background was initially colorless, whereas upon untreated strips, the background was initially orange. The resolution of a mixture of compounds was improved, in most instances.

The addition of acetic acid as the third component to a *n*-butanol-water solvent system resulted in

R_f values regardless of the solvent system used. On the other hand, compounds such as doxylamine succinate, pyrimilamine maleate, and pheniramine maleate exhibited generally lower R_f values and the magnitude of R_f was found to be dependent to a considerable extent upon the solvent system used. The R_f values of pyrazinazine hydrochloride varied between 0.74 and 0.96; those of methapyrilene hydrochloride between 0.19 and 0.73. This characteristic is useful as a guide to the selection of a resolving system.

REFERENCES

- (1) Kleckner, L. J., and Osol, A., *THIS JOURNAL*, 44, 762 (1955).
- (2) Baues, D., *J. Assoc. Offic. Agr. Chemists*, 34, 703 (1951).
- (3) Bingenheimer, L. E., Jr., and Christian, J. E., *THIS JOURNAL*, 40, 164 (1951).
- (4) Osol, A., and Sideri, C. N., *ibid.*, 44, 761 (1955).
- (5) Jones, H. M., and Brady, E. S., *ibid.*, 38, 579 (1949).
- (6) Blaug, S. M., and Zopf, L. C., *ibid.*, 45, 9 (1956).
- (7) Munier, R., and Machehoeuf, M., *Bull. Soc. Chim. Biol.*, 33, 846 (1951).

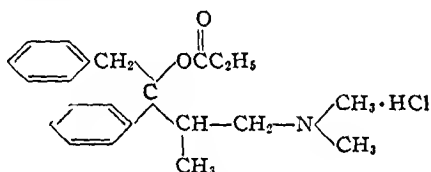
Notes

A Note on the Crystallography of *d*-Propoxyphene Hydrochloride*

By HARRY A. ROSE

A NEW COMPOUND, *d*-propoxyphene hydrochloride, prepared at the Lilly Laboratories has about the same analgesic response as codeine phosphate (1). The chemistry of the compound is discussed elsewhere (2, 3). Data are given here which permit the identification of this compound by crystallographic methods.

Chemically, *d*-propoxyphene hydrochloride is α -*d*-4-dimethylamino-1,2-diphenyl-3-methyl-2-propionyloxybutane hydrochloride and has the following structure



EXPERIMENTAL

Crystallization from ethyl acetate resulted in orthorhombic prisms elongated parallel to the *b* axis. The crystals showed the prism {110} and the orthodome {101}. Measurements of the interfacial angles gave the following results: 110 > 110 (polar) 94° 18' (optical), 93° 58' (X-ray), 101 > 101 (polar) 86° 03' (optical), 86° 10' (X-ray).

The X-ray powder diffraction data were obtained using copper radiation and nickel filter with a camera 114.6 mm in diameter. A wavelength value of 1.5405 Å was used in the calculations. The indexing of the powder pattern was done on the basis of single crystal rotation patterns around both the *b* and *c* axes.

On heating, *d*-propoxyphene hydrochloride melts in the range 168–171°. The melt does not crystallize on cooling.

TABLE I.—X-RAY CRYSTALLOGRAPHIC DATA

Unit Cell Dimensions	$a_0 = 12.83 \text{ Å}$, $b_0 = 13.75 \text{ Å}$, $c_0 = 12.00$
Formula Weight per Cell	4
Formula Weight	375.9
Density	1.173 Gm./cc (floatation), 1.181 Gm./cc. (x-ray).
Axial Ratio	$a:b:c = 0.9331 : 1.0872$
Space Group	$P2_12_12_1$

TABLE II.—OPTICAL CRYSTALLOGRAPHIC DATA

Refractive Indices.	(5893 Å, 25°). $\alpha = 1.560$
	$\beta = 1.582$, $\gamma = 1.638$
Optic Axial Angl.	(+) $2V = 66^\circ$ (calcd. from α , β and γ).
Optic Axial Plane.	100
Acute Bisectrix.	$\gamma = c$

TABLE III.—X-RAY POWDER DIFFRACTION DATA

<i>d</i>	<i>I</i> / <i>I</i> ₁	<i>h k l</i>	<i>d</i> (calcd)
9.50	0.03	110	9.38
8.79	0.33	101	8.76
7.40	0.20	111	7.39
6.39	0.03	200	6.41
6.02	1.00	120, 002, 021	6.06, 6.00, 5.97
5.60	0.03	201	5.66
5.38	0.03	121	5.41
5.06	0.27	112	5.05
4.55	0.20	022	4.52
4.38	0.20	202, 221	4.38, 4.37
4.08	0.67b	212, 310, 131, 301	4.17, 4.08, 4.06, 4.03
3.86	0.07	311	3.87
3.76	0.20	230	3.73
3.65	0.07	320	3.63
3.50	0.20	132, 302, 321	3.50, 3.48, 3.47
3.35	0.07	123	3.34
3.21	0.13	400, 141	3.21, 3.20
3.10	0.07	401	3.10
3.01	0.07	240, 331, 411	3.03, 3.03, 3.01
2.92	0.13	303, 142	2.92, 2.91
2.86	0.07	313	2.86
2.78	0.03	332	2.77
2.70	0.03	204	2.72
2.62	0.07	151, 341	2.62, 2.61
2.52	0.13	224, 510	2.53, 2.52
2.46	0.07		
2.40	0.03		
2.34	0.07b		
2.27	0.03		
2.23	0.03		
2.14	0.03b		
2.09	0.03b		
2.02	0.03		
2.00	0.03		
1.95	0.03		
1.89	0.03		

REFERENCES

- (1) Gruber, C. M., et al., *J. Pharmacol. Exptl. Therap.*, **118**, 280 (1956).
- (2) Pohland, A., and Sullivan, H. R., *J. Am. Chem. Soc.*, **75**, 4458 (1953).
- (3) *Ibid.*, **77**, 3400 (1955).

* Received July 1, 1957 from the Analytical Department, Eli Lilly and Company, Indianapolis, Ind.

A Note on Specific Rotation and Temperature Coefficient*

By JOSEPH ROSIN and C. J. WILLIAMS

IT IS UNIVERSALLY accepted that the temperature at which it is determined affects optical rotation as it does other physico-chemical properties, e. g., specific gravity, refractive index, etc. For this reason numerical data of these properties are either "suffixed" with the temperature at which the data apply or were measured, or it is implied by specific statements in the introductory remarks. In the United States Pharmacopeia and the National Formulary the temperature for measuring rotation is 25°; in the British Pharmacopoeia and the majority of European Pharmacopoeias it is 20°.

In the past several years the senior author has had correspondence with several laboratories about reported divergences in the specific rotation between laboratories on the same samples. This situation came to focus in a collaborative study of the purity of an amino acid. Discrepancies in the reports on the specific rotation were of a puzzling magnitude. Normal variations in concentration, in the authors' experience even as much as 50%, would not account for the differences. Nor could they be attributed, so it was thought, to normal variations in laboratory temperatures. In chemical laboratories it is generally assumed that a deviation of $\pm 2-3^\circ$ from the standard temperature has no significant effect on specific rotation value. As a matter of last resort we decided to check experimentally the effect of temperature variations within approximately the range of usual laboratory temperatures.

The solution of the chemical in a 100 mm polarimeter tube fitted with a thermometer was cooled to the low temperature (20° or 22°) and the rotation measured by taking the mean of 6 to 10 readings. The temperature of the solution was then raised by warming one or two degrees and the rotation determined as before described. After observing the rotation at the highest temperature (30°) the solution was cooled to the original lowest temperature and the rotation again determined at this temperature. This was done to make sure that no deterioration of the chemical had occurred during the intervals. The first value found at the lowest temperature and that of the check did not deviate from each other by at most one degree of specific rotation.

The temperature "coefficient"—the increase or decrease in specific rotation per degree difference in temperature—was thus found surprisingly large for the chemical first investigated, and when the reported values by the several laboratories were corrected for the temperature at which they were determined by the coefficient we established, the results were harmonious.

This finding induced us to test several other medicinal chemicals for their specific rotation temperature coefficient. See the results in Table I.

The specific rotation values in the Table are on the anhydrous basis. The solvent, when not otherwise indicated in the last column, is water.

Of the 16 specimens thus examined, 6 showed a not insignificant temperature coefficient, and half of them a rather large one. This study emphasizes what is well known in science that assumptions are not to be relied upon.

TABLE I — SPECIFIC ROTATION-TEMPERATURE COEFFICIENTS OF CERTAIN MEDICINAL CHEMICALS

	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	Difference	Temp. Coeff.	Concn.
L-Arginine HCl	+21 8°			21 6°	21 3°	21 3°	21 4°	21 4°	21 3°	21 1°	21 2°	0 6°	0 06°	5 ^b
Ascorbic Acid			+21 4°	21 3°	21 3°	21 3°	21 3°	21 3°	21 3°	21 1°	21 1°	0 3°	0 03°	10
Cinchonine			+230°	229°	229°	229°	229°	229°	228°	228°	227°	3 0°	0 33°	1°
Cocaine Sulfate	-113 4°	436 1°	113 4°	113 2°	113 2°	113 2°	112 9°	112 9°	112 9°	112 9°	112 9°	0 5°	0 05°	2
Cocaine	-439 7°	212 0°	432 4°	428 3°	422 0°	421 2°	414 7°	414 7°	412 6°	410 4°	409 0°	30 7°	2 8°	1
L-Cystine	-213 9°		210 7°	209 5°	208 7°	206 7°	204 5°	202 7°	200 4°	199 3°	199 3°	14 6°	1 46°	3 5 ^d
Dehydrochloric Acid			+31 0°	31 0°	30 8°	30 8°	31 3°	31 0°	31 0°	31 0°	31 0°	0 0°	0 0°	2 ^d
Dextrose			+53 0°	52 6°	52 6°	52 8°	52 6°	52 6°	52 8°	52 8°	52 6°	0 4°	0 04°	5
Dihydrostreptomycin SO ₄		-86 8°	86 6°	86 4°	86 2°	86 2°	86 2°	86 2°	85 8°	85 8°	85 6°	1 2°	0 12°	5
Ephedrine Hydrochloride		-34 2°	34 2°	34 2°	34 2°	34 0°	34 0°	34 2°	34 0°	34 0°	33 8°	0 4°	0 04°	5
Ephedrine Sulfate		-31 2°	31 2°	31 2°	31 0°	31 0°	31 0°	31 0°	31 0°	31 0°	31 0°	0 2°	0 02°	5
Hydrocortisone Acetate	+168 3°	167 8°	167 3°	166 5°	165 8°	165 8°	164 8°	164 8°	164 5°	164 5°	164 5°	3 8°	0 34°	4
L-Methionine	-7 9°		7 8°	7 6°	7 6°	7 5°	7 5°	7 5°	7 2°	7 1°	7 1°	0 8°	0 07°	4
Penicillin Sodium	+296 8°	294 9°	293 4°	292 1°	291 1°	290 8°	289 5°	287 8°	287 8°	287 3°	286 9°	9 9°	0 9°	3
Phocarpin Nitrate			+82 3°	82 2°	81 7°	81 0°	80 7°	80 2°	80 0°	79 7°	79 5°	2 8°	0 3°	2
Strychnine Sulfate			-27 8°	27 5°	27 1°	27 1°	27 1°	26 6°	26 6°	26 5°	26 2°	1 6°	0 18°	7 5

* Difference in degrees of specific rotation determined at highest and lowest temperatures. ^b In 1 N HCl. ^c In EtOH + CHCl₃. ^d In Dioxane.

* Received November 15, 1957 from the Laboratories of Merck & Co., Rahway, N. J.

Book Notices

Lo Rauwolfia By LIVIO MEDIANI Casa Editrice Ambrosiana, Milan, 1957 xvi + 582 pp 15 5 x 22 cm

This book reviews, in a competent manner, the history, chemistry, and pharmacology of the several known species of *Rauwolfia*. It includes references to the original literature, but has no index.

Science looks at Smoking A New Inquiry into the Effects of Smoking on Your Health By ERIC NORTHROP Coward McCann, Inc., New York, 1957 190 pp 13 5 x 20 5 cm Price \$3

An effort is made to inquire into the effects of smoking, and to appraise some of the reports that have been so widely circulated. The reports on smoking as a cause of lung cancer and heart failure are reviewed and evaluated. The author points out that faulty research methods and preconceived attitudes have made some of the publicized conclusions invalid and suspect, and that as a result the public has been unduly alarmed by the hasty release of inconclusive reports and findings.

Tables for Use with Binomial Samples By DONALD MAINLAND, LEE HERRERA, and MARION I. SURCLIFFE Department of Medical Statistics, New York University, New York, 1957 ix + 83 pp 27 5 x 21 cm Price \$2

The statistical tables published by Mainland and his collaborators during the past eight years are of pharmaceutical interest. All of the tables, with instructions, are now reissued in book form by photoffset from typed material, with a stiff (twillpex) cover and spiral binding. It contains many more entries than in the original versions, in order to minimize the need for interpolation. The tables remove the need for computation or reduce it to a line or two of simple arithmetic. Tables I-IV are for the significance testing of fourfold contingency tables (comparison of two percentage frequencies). Tables V-IX show binomial confidence limits for sample sizes from 1 to 100,000, with intervals of 1% or less between sample percentages for the 95% and 99% limits, and somewhat wider intervals for the 80% limits. Estimates of sample sizes required under various conditions are obtainable from all the tables, but Table X is specially designed for this.

Veterinary Toxicology (Formerly Lander's Veterinary Toxicology) By R. J. GARNER The Williams & Wilkins Company, Baltimore, 1957 (distributors for Bailliere, Tindall and Cox, London) iv + 415 pp 15 x 23 cm Price \$7 50

This book was published originally under the title "Lander's Veterinary Toxicology," the last edition of which appeared in 1945. Since that time, so much new material has accumulated that it has been necessary to prepare a complete revision. Most toxic compounds to which animals may be exposed have been covered by dividing them into six classifications. These classifications include in-

organic substances, organic compounds (I) drugs (II) pesticides, and (III) miscellaneous. In two other chapters poisonous plants and radioactive materials are discussed from a toxicological standpoint. The eighth and final chapter covers quite adequately methods employed in toxicological analysis.

Volumetric Analysis, Vol. III-Titration Methods Oxidation-Reduction Reactions By I. M. Kolthoff and R. Belcher with the cooperation of V. A. Stenger and G. Matsuyama Interscience Publishers, Inc., New York, 1957 ix + 714 pp 15 x 23 cm Price \$15

The third and last volume of "Volumetric Analysis" deals exclusively with oxidation-reduction titrations. It is logically arranged in a series of fifteen chapters, is well documented throughout, and is provided with author and subject indexes. The first chapter discusses reactions, indicators, and general techniques in oxidation-reduction titrations. Other chapters discuss potassium permanganate as a volumetric reagent, iodimetry with permanganate and with ceric salts, potassium dichromate as oxidizing titrant, iodometry, determination of water with the Karl Fischer reagent. Other chapters cover the use of potassium iodate as an oxidizing titrant, oxidation of organic compounds with periodate, potassium bromate as a titrimetric reagent, oxidation with hypohalites, titrations with iron, titanium, and other strong reducing agents, and miscellaneous titrations. The book constitutes a useful reference to titration methods involving oxidation-reduction reactions.

Advances in Pest Control Research Vol. I Edited by R. L. METCALF Interscience Publishers, Inc., New York, 1957 vii + 514 pp 15 x 23 cm Price \$11

This is the first in a series of selected contributions by specialists in fields related to pest control research. The pests include economically destructive species of plants and animals, variously classified as viruses, bacteria, fungi, weeds, protozoa, nematodes and other helminths, molluscs, insects and other arthropods, fish, birds, and rodents. The text of this volume includes the following subjects: Control of health hazards associated with the use of pesticides, The chemistry and mode of action of herbicides, Uses of radioisotopes in pesticide research, The chemistry of action of organic phosphorus insecticides, Mechanisms of fungitoxicity, Recent advances in control of soil fungi, Repellents for biting arthropods, The status of systemic insecticides in pest control practices, Chemical analysis of pesticide residues, and Bioassay of pesticide residues. The text includes many references which are given at the end of each chapter. A good subject index is appended.

The style, format, type, and binding of the book are very good. The text material represents good, comprehensive reviews with suggested approaches to

further research. The book should be very useful in public health, pharmacy, chemistry, agriculture, and analytical libraries.

Basic Facts of Pharmacology. By S. M. BROOKS. W. B. Saunders Co., Philadelphia, Pa., 1957. 323 pp. 14 x 20.5 cm. Price \$4.

This textbook is designed to present the essential facts of pharmacology to the members of the nursing profession—especially student nurses. The author claims little originality for most of the text except the organization and literary style. The latter are excellent. Included is a tabulation of important drugs in current use—giving generic name, common or trade name, U.S.P., N.F., or N.N.R. listing, and classification, use or condition treated. A glossary and a general index are appended.

Basic Pharmacology for Nurses. By JESSIE E. SQUIRE. The C. V. Mosby Co., St. Louis, 1957. 265 pp. 17.5 x 25.5 cm.

This book is a good text for teaching the required pharmacology to nurses. The arrangement of material is intended to aid in memorizing the information, and the "assignments" include good review questions. A glossary of frequently used terms and an index are included.

Cosmetics: Science and Technology. Edited by EDWARD SAGARIN. Interscience Publishers, Inc., New York, 1957. xix + 1,433 pp. 16.5 x 24.5 cm. Price \$25.

This comprehensive compilation of technical information on cosmetics and practical formulations is a very useful addition to the literature in its field. The text is divided into four major divisions headed: The scope of cosmetics, Toilet preparations (about 800 pages), Manufacture and technology, Physiological considerations, and Legal considerations.

The similarity between cosmetic formulations and pharmaceutical lotions, ointments, aerosols, etc., make this book a very valuable reference for the pharmacy as well as the cosmetic laboratory. The text is documented with references that are given at the end of each chapter. An index of trade names of materials used in cosmetic formulations, a list of suppliers, an author index, and a subject index are appended.

The type, format, printing, and binding are very good in this book that should find a place in many laboratories and libraries.

The Dermatologist's Handbook. By ASHTON L. WELSH. Charles C Thomas, Publisher, Springfield, Ill., 1957. xii + 427 pp. 21.5 x 28 cm. Price \$15.

This book represents an attempt to organize into a rational systematic classification, for purposes of instruction and reference, the topical and internal therapeutic agents concerning which a dermatologist must have knowledge. It is noted that synthetics, detergents, excitants, irritants are with us and about us; and that the dermatologist is confronted daily with manifestations of sensitivity to external agents and to internal agents consumed voluntarily or prescribed by other physicians.

The book is an arrangement utilizing pharmaceutical dosage forms and pharmacologic classifications for external preparations and therapeutic indications for grouping preparations used internally.

Although the compilation is not intended to cover all classes of drugs completely, there are enough "representative compositions" to give the impression that the title "Dermatologist's Formulary" might be more suitable for this volume. The information, which is presented in abstract monograph form, should be useful as a ready reference. The book is very well printed, has a good format and clear type, and has a good binding. An index of manufacturers and distributors and a subject index are appended.

Hormonal Regulation of Energy Metabolism. Compiled and edited by LAURANCE W. KINSELL. Charles C Thomas, Publisher, Springfield, Ill., 1957. xii + 242 pp. 15 x 23 cm. Price \$5.25

This book records the papers and discussions of a Conference on Hormonal Regulation of Energy Metabolism. Subjects included are: Certain aspects of hormonal regulation of carbohydrate metabolism, Hormonal regulation of enzymatic activity, The anterior pituitary in relation to energy metabolism, The mechanism of the influence of pituitary growth hormone on metabolism, The thyroid in relation to energy metabolism, Insulin—reminiscences, Diabetes and the insulin problem, and The adrenal cortex and energy metabolism. The importance of insulin in overall energy metabolism is stressed by its frequent inclusion in discussions of all phases of the problem.

The book has a good style, with excellent type, format, and binding. Its one shortcoming is the absence of a general index, which detracts from its usefulness as a reference volume.

Rogers' Inorganic Pharmaceutical Chemistry. 6th ed. By TAIRO O. SOINE and CHARLES O. WILSON. Lea & Febiger, Philadelphia, 1957. 705 pp. 15.5 x 23.5 cm. Price \$9.50

The textbook, that was originally written by Charles H. Rogers, has again been revised to be used readily with U. S. P. XV and N. F. X by students and practitioners of pharmacy. Aside from the modernization of the text material, changes from the 5th ed., which was reviewed in *THIS JOURNAL*, 41, 339(1952), include: deletion of discussions of non-official compounds of interest only from a chemical standpoint; deletion of discussions of pharmaceutical preparations in which official inorganic compounds occur, and provision of a statement on the purpose of these compounds in each preparation. The text follows closely the Periodic Table arrangement of the elements.

The text, type, format, and binding are very good. The inclusion of the kind of information that has been useful and helpful in the past assures the continued popularity of this book.

Pharmacology and Oral Therapeutics. 11th ed. By EDWARD G. DOBBS. The C. V. Mosby Company, St. Louis, 1957. 579 pp. 14 x 22 cm. Price \$9.

This is the 11th edition of a well-known textbook

formerly published under the title "Pharmacology and Dental Therapeutics." It now contains new material pertinent to the field of oral medicine. It covers many new drugs that have appeared since 1951 and the descriptive material conforms to the current revisions of the United States Pharmacopeia and the National Formulary.

Chemical Properties of Organic Compounds, an Introduction. By ELLIOT N. MARVELL and ALBERT V. LOGAN. John Wiley & Sons, Inc., New York, 1957. x + 326 pp. 14.5 x 23 cm. Price \$4.75.

This book uses theoretical organic chemistry as a basis for teaching introductory organic chemistry. Reactions have been chosen to emphasize principles and reduce the reader's memory burden. The concept of studying "functional groups" is stressed, and special topics are limited to those involving chemistry that will be useful to those this book was designed to serve.

Textbook of Pharmacognosy. 7th ed. By GEORGE EDWARD TREASE. The Williams and Wilkins Company, Baltimore, 1957. viii + 806 pp. 13.5 x 21.5 cm. Price \$8.50.

The 7th edition of this standard textbook by Professor Trease has been thoroughly revised and includes more information than heretofore on the newer physical and chemical methods of drug evaluation. The botanical side of pharmacognosy, however, has not been deemphasized.

Aids to Materia Medica and Therapeutics. 5th ed. By J. W. HADGRAFT. Bailliere, Tindall and Cox, London, 1957, (The Williams and Wilkins Company, Baltimore). viii + 259 pp. 10 x 16 cm. Price \$3.25.

This little book is designed to indicate to the medical student the way in which drugs are formulated for use in therapeutics and the alternative preparations available. It has been completely revised in accordance with the British Pharmacopeia of 1953 and the Addendum 1955.

The Chemistry of Natural Products. Vol. I, *The Alkaloids.* Edited by K. W. BENTLEY. Interscience Publishers, Inc., New York, 1957. vii + 237 pp. 15 x 23 cm. Price \$4.

Important facts about the structural chemistry of alkaloids and the degradative and synthetic means whereby the structures were elucidated are presented and illustrated by a profusion of formulas.

Ten Million and One, Neurological Disability as a National Problem. By the Arden House Conference sponsored by the National Health Council. Edited by ALICE FITZ GERALD and JUSURUS J. SCHIFFERES. Hoeber-Harper, New York, 1957. xv + 102 pp. 13.5 x 20.5 cm. Price \$3.50.

This book explores possibilities of meeting the problems posed by neurological disabilities and illustrates how a cooperative approach to the problem can hope to yield better results in diagnosis, treatment, management, education, and vocational placement of the neurologically disabled. It is of interest

to everyone professionally or personally concerned with neurological disabilities.

Manual of Nutrition. 4th ed. Prepared by the Adviser's Division (Food) of the Ministry of Agriculture, Fisheries and Food. First edition by Magnus Pyke. Printed by Fosh and Gross Ltd., London, for Philosophical Library, New York, 1957. 70 pp. 15 x 24 cm. Price \$3.50.

This is the 4th edition of a book first published in Great Britain in 1945. It presents the principles of nutrition and good, healthy feeding.

V. N.

The Chemistry of Organic Medicinal Products. 4th ed. Edited by GLENN L. JENKINS, WALTER H. HARTUNG, KENNETH E. HAMLIN, JR., and JOHN B. DATA. John Wiley and Sons, Inc., New York, 1957. x + 569. 15.5 x 23.5 cm. Price \$10.75.

This new edition shows an extensive revision of the material in the third edition, thus bringing the subject matter well up to date. New material contained in the revised work includes a chapter on antibiotics and an increased section on hormones. All references, where possible, to the *in vivo* action of drugs are given and many new compounds of pharmaceutical interest are discussed. In this revision, much of the elementary organic chemistry and material on little used compounds have been deleted, thus making room for the newer material without appreciably enlarging the text.

Introduction to Protein Chemistry. By SIDNEY W. FOX and JOSEPH F. FOSTER. John Wiley & Sons, Inc., New York, 1957. viii + 459 pp. 15 x 23 cm. Price \$9.50.

This book covers the fundamental aspects of protein chemistry and indicates ways in which the knowledge is basic to biology, nutrition, and food technology.

The Lynn Index. A Bibliography of Phytochemistry (Monograph I). Organized and edited by JOHN W. SCHERMERHORN and MAYNARD G. QUIMBY. Massachusetts College of Pharmacy, Boston, 1957. 46 pp. 15 x 23 cm. Price \$1.

The late Dr. Eldin V. Lynn, Chairman of the Department of Chemistry at the Massachusetts College of Pharmacy, spent many years searching the literature for references to phytochemistry. This collection has come to be known as the "Lynn File." The organizing and editing of the material for publication as *The Lynn Index* was started in June 1957. It is estimated that seventy or eighty issues will be needed to complete the project. The contents of each monograph will be so arranged that one can determine what work has been reported on a given plant and what constituents have been isolated and identified. Each monograph will be concerned with species from a single plant family or from a group of related families. The contents of Monograph I deal with the order *Centrospermae* and include nearly 400 references to approximately sixty genera distributed among the following families: *Aizoaceae*, *Amaranthaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Nyctaginaceae*, *Phytolaccaceae*, and *Portulacaceae*.

ANNOUNCEMENT

This issue of the *Scientific Edition* of the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION is supplied in two parts. Part I is the customary edition of the JOURNAL, and Part II is a supplement carrying the list of active members of the AMERICAN PHARMACEUTICAL ASSOCIATION, the list of current ASSOCIATION officers, a brief history and summary of the ASSOCIATION's activities, the list of officers of the ASSOCIATION and places of meeting since its organization, the Constitution and By-Laws of the ASSOCIATION and the By-Laws of the Council, the House of Delegates and the Sections, the Certificate of Incorporation, the Code of Ethics, the lists of national, state and local pharmaceutical organizations with the names of their Secretaries, the list of accredited Colleges of Pharmacy, State Boards of Pharmacy, and other similar information.

Prior to 1945, the PROCEEDINGS of the AMERICAN PHARMACEUTICAL ASSOCIATION were published either separately or in installments in the monthly JOURNAL. Since 1949, we have been issuing the information referred to in the previous paragraph as a supplement to the *Scientific Edition* of the JOURNAL so that it could be available as a separate volume to those who refer to this information frequently. The PROCEEDINGS of the Annual Convention are published in narrative form in the *Practical Pharmacy Edition* of the JOURNAL immediately following the annual convention.

The PROCEEDINGS of the 1954 and 1955 conventions were published in narrative form in the September, 1954 and June, 1955 numbers respectively of the *Practical Pharmacy Edition* of the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION and continued in subsequent editions of the JOURNAL as far as the addresses and reports of special interest are concerned. Many of the scientific papers and those dealing with practical pharmacy, economics, history, education, and legislation which were presented at the various section meetings have been or are being published in various issues of either the *Scientific* or *Practical Pharmacy Editions* of the JOURNAL.

The membership list, which is a part of the supplement to the March, 1958, *Scientific Edition*, includes active members in good standing as of December 31, 1957, and also those members who were not listed in any previous printed list but who may not have continued to be in good standing up to December 31, 1957. Such arrangements have been made to assure continuation of availability of a published list of all members in one of the ASSOCIATION publications since its origin. Members who joined the ASSOCIATION after January 1, 1958, have been listed in one of the issues of the *Practical Pharmacy Edition* of the JOURNAL appearing since that date, and their names will, of course, be included in the next membership list to be published.

Members of the ASSOCIATION are earnestly requested to check the accuracy of the address given with their names and send us any necessary changes.

Robert P. Fischelis, *Secretary*
AMERICAN PHARMACEUTICAL ASSOCIATION

BRIEF HISTORY AND SUMMARY OF THE ACTIVITIES OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

The AMERICAN PHARMACEUTICAL ASSOCIATION was formally organized in Philadelphia, October 6-8, 1852, following a preliminary meeting of leaders of the profession in New York City in 1851. Perhaps the most important factor leading to the formation of the organization was a desire on the part of the pharmacists and physicians of that day to improve the quality of drugs which were being imported. The leaders in the movement to organize the ASSOCIATION were practicing pharmacists, members of faculties of Colleges of Pharmacy, and other persons of scientific training who were anxious to maintain and achieve higher standards of professional practice and improvement of the quality of drugs offered to the American people.

The objectives as stated in the Constitution were to "unite the educated and reputable pharmacists of America in an effort to improve and regulate the drug market by preventing the importation of inferior, adulterated, or deteriorated drugs, and by detecting and exposing home adulterations, to encourage proper relations with the other health professions; to improve pharmaceutical science by diffusing scientific knowledge, fostering pharmaceutical literature, developing talent, stimulating discovery and invention, and encouraging domestic production and manufacture of drugs and medicines; to regulate education and training of pharmacists; to suppress empiricism and to restrict the dispensing and distribution of medicines to qualified pharmacists; to uphold standards of authority in the education, theory, and practice of pharmacy with a view to the highest good and the greatest protection to the public."

The ASSOCIATION's membership includes practicing pharmacists, wholesale druggists, pharmaceutical manufacturers and their representatives, drug importers, teachers, research workers, editors and publishers of pharmaceutical literature, pharmaceutical chemists, food and drug officials, hospital pharmacists, association officials, students, and pharmacists in the government service.

Annual dues for Active Members are \$15, including the *Practical Pharmacy Edition* of the JOURNAL. Associate Membership is open through Student Branches to students in accredited schools and colleges of pharmacy. Such members have all the privileges of active membership except those of voting, holding office, or serving on standing committees of the ASSOCIATION. They enjoy full privileges of voting and holding office in Student Branches of which they are members.

Associate (student) member dues are \$3.00 a year, including the *Practical Pharmacy Edition* of the JOURNAL or the *Scientific Edition* of the JOURNAL; or \$5.00 a year when both editions of the JOURNAL are included. Of these amounts, \$1.00 is remitted to the Student Branch for its support.

The by-laws also provide that any person approved by the Council as meeting the qualifications for active membership in the ASSOCIATION may be granted life membership if he pays to the Treasurer the sum of \$250 during the first ten years of his connection therewith and also to any active member, not in arrears, who after 10 years shall pay the sum of \$200, or after 15 years the sum of \$150, or after 20 years the sum of \$120 or after 25 years the sum of \$75. Any member who may have paid annual dues for 40 consecutive years, may become a life member upon approval by the Council.

Separate subscription to the JOURNALS is \$10.00 a year for the *Scientific Edition* of the JOURNAL and \$5.00 a year for the *Practical Pharmacy Edition* of the JOURNAL or \$14.00 a year for both editions.

Publications of the ASSOCIATION include the National Formulary, which is now in its tenth edition; the Pharmaceutical Recipe Book, now out of print; the *Scientific Edition* of the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, now in its forty-seventh volume; the *Practical Pharmacy Edition* of the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, now in its nineteenth volume; and *Drug Standards*, formerly known as the *Bulletin of the National Formulary Committee*, now in its twenty-sixth volume. The ASSOCIATION also publishes monographs on selected subjects at irregular intervals.

The ASSOCIATION holds annual meetings. The ASSOCIATION's 33 Local Branches and 76 Student Branches in various cities hold monthly or semi-monthly meetings.

The ASSOCIATION makes two scientific awards: The Ebert Prize, consisting of a medal, awarded annually to the author of the best paper published in the *Scientific Edition* of the JOURNAL; and The Kilmer Prize, consisting of a gold key, awarded annually for the best paper submitted by a Senior Student in a College of Pharmacy on a subject related to pharmacognosy.

At the request of the Chilean Iodine Educational Bureau Inc., the ASSOCIATION has acted as sponsor of an Award for Research in the Pharmaceutical Chemistry of Iodine. This is an award of \$1,000 given in recognition and encour-

agement of outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The New York Branch of the AMERICAN PHARMACEUTICAL ASSOCIATION also makes an annual award known as The Remington Honor Medal to the man or woman who has done the most for American pharmacy during the preceding year or whose efforts during a number of years have resulted in the most important contribution to American pharmacy.

The ASSOCIATION is governed between annual conventions by the Council consisting of sixteen members, and its principal business at annual meetings is carried on by the House of Delegates which is made up of representatives of State and National Pharmaceutical Associations, Sections of the ASSOCIATION, the Council and the Past Presidents.

The ASSOCIATION participates actively in the American Council on Pharmaceutical Education, the American Foundation for Pharmaceutical Education, American Documentation Institute, the U. S. Pharmacopeial Convention, the American Association for the Advancement of Science, the National Health Council, the International Pharmaceutical Federation and the Pan-American Pharmaceutical Federation and other groups.

Its representatives are often called into consultation by various Government agencies on matters pertaining to pharmaceutical education; manpower; and production, standardization, and distribution of drugs and medicines. It also maintains a close liaison with the armed forces and with other professional and scientific associations, foundations and conferences.

The activities of the laboratories of the ASSOCIATION are directed toward the establishment and improvement of standards of purity, quality, and strength of drugs and medicines, particularly those of the National Formulary. They also include researches and routine procedures associated with prescription problems, establishment of tolerances for extemporaneous compounding, dispensing techniques and the development of dosage forms for drugs.

Through its Committee on Pharmaceutical Research the ASSOCIATION makes research grants for studies relating to the standardization and development of drugs.

The ASSOCIATION maintains its own building and laboratory, library and museum facilities in Washington. The building and grounds, including the memorial to all pharmacists who served in the wars of our country, occupy the block on Constitution Avenue between 22nd and 23rd Streets. This building also houses the editorial and administrative offices of the ASSOCIATION.

The members of the AMERICAN PHARMACEUTICAL ASSOCIATION function as individuals in the general sessions of the ASSOCIATION at the na-

tional convention. The general sessions may take final action on any item of business acted on by the House of Delegates.

The reports of all Officers and Committees and of the Council are referred to the House of Delegates and the House takes action on recommendations offered from these sources and from individual members of the House. Usually such action is taken through the Committee on Resolutions, which submits its recommendations to the House. The recommendations are then debated and finally acted upon. They must be approved at the final general session of the ASSOCIATION before they become the policy of the ASSOCIATION on the subject covered.

The Council of the ASSOCIATION acts for the House of Delegates and for the ASSOCIATION in the interim between national conventions. Since the Secretary of the ASSOCIATION is also the Secretary of the House of Delegates and of the Council and is designated as the General Manager of the ASSOCIATION, it becomes his duty, as the Administrative Officer, to implement the actions taken by the ASSOCIATION with the help of the other officers, committees and the staff.

The varying interests of the members of the AMERICAN PHARMACEUTICAL ASSOCIATION are provided for at the convention through meetings of the Sections of the ASSOCIATION. There are six Sections, namely, the Scientific Section, the Section on Practical Pharmacy, the Section on Education and Legislation, the Section on Pharmaceutical Economics, the Section on Historical Pharmacy and the Pharmacy Student Section. These sections provide opportunities for the presentation of papers and discussions on a wide variety of subjects.

There are also a number of affiliated and related organizations which meet either immediately prior to, or subsequent to, the annual meetings of the AMERICAN PHARMACEUTICAL ASSOCIATION. Of these, the American Society of Hospital Pharmacists and the American College of Apothecaries are affiliated organizations, which means that membership in these Associations is limited to persons holding membership in the A. Ph. A.

The ASSOCIATION also has affiliated with it the Women's Auxiliary of the A. Ph. A., which is an organization of wives and other female relatives of male members of the parent Association, organized to assist in carrying out the A. Ph. A. objectives. Although it is principally a social organization, the objectives of the Auxiliary include development of scholarship funds and other activities in the interest of women pharmacy students.

As the ASSOCIATION enters its 106th year of activity it is geared to the presentation of a well-rounded program designed to sponsor and carry out activities in the field of health and medical care, which will serve the best interests of the public and the profession.

CONSTITUTION AND BY-LAWS OF THE American Pharmaceutical Association

CONSTITUTION

(With all amendments to December 31, 1957)

Article I. Name and Seal. This ASSOCIATION shall be called the "AMERICAN PHARMACEUTICAL ASSOCIATION" The ASSOCIATION shall have an official seal.

Article II. Objects. This ASSOCIATION shall exist for the following purposes:

1. To improve and promote the public health by aiding in the establishment of satisfactory standards for drugs, and to aid in the detection and prevention of adulteration and misbranding of drugs and medicines, and to take such steps as an ASSOCIATION and in cooperation with other organizations as will assure the production and distribution of drugs and medicines of the highest quality.

2. To foster and encourage interprofessional relations to the end that pharmacists, physicians, and members of other allied professions may contribute to the promotion of the public health and welfare in fullest measure.

3. To improve the art and science of pharmacy for the general welfare of the public by fostering the publication of scientific information relating to the practice of pharmacy, and by the preparation and distribution of publications which will record the progress of pharmacy and aid in the development and stimulation of discovery and invention and in interesting competent personnel in the practice of pharmacy as a career.

4. To provide a system of education and training in the art of pharmacy, calculated to produce competent personnel for all phases of the practice of pharmacy and the training of pharmacists as a means of providing the greatest protection for the public at large.

5. To support a system of licensure and registration of pharmacists which will assure to the public the availability of competent personnel to discharge the accepted functions of the practice of pharmacy and assure the availability of pharmacists and pharmaceutical service which will provide at all times for the distribution of drugs and medicines under the supervision of qualified pharmacists.

6. To develop, maintain, and enforce a Code of Ethics which will assure to the public the highest type of pharmaceutical service, safeguard the professional relations between medical practitioners, pharmacists, and patients, and develop intrapro-

fessional relations which will tend to uplift the profession scientifically, spiritually and morally.

7. To cooperate to the fullest extent in conducting research examinations, investigations, experiments, and in the dissemination of information in the field of pharmacy with agencies of the United States Government, such as the United States Public Health Service, The Surgeons General of the Army and Air Force, The Bureau of Medicine and Surgery of the Navy, The Department of Medicine of the Veterans Administration and similar agencies of States and Territories of the United States. The ASSOCIATION may accept grants in aid and other forms of payment for the expenses of conducting such research, examinations, investigations and experiments as it may be requested to make by the Congress of the United States, or any agency of the United States Government, or the Government of any State or Territory of the United States.

Article III. Membership. This ASSOCIATION shall consist of active, associate, life and honorary members, and shall hold its meetings annually.

Article IV. Headquarters—American Institute of Pharmacy. Implementation of the aims and objects of the AMERICAN PHARMACEUTICAL ASSOCIATION shall be accomplished through a headquarters organization established and housed in the American Institute of Pharmacy.

Article V. Officers. The officers of the ASSOCIATION shall be a president, two vice-presidents and an honorary president, who shall be elected for a term of one year, and a secretary, a treasurer and nine councilors, who shall be elected for a term of three years. The officers shall be elected as provided in the By-Laws and shall hold office until the installation of their successors.

Article VI. Organization. The functions of the ASSOCIATION are performed by the members through the agency of the General Sessions, the House of Delegates, the Council, the Sections, and the Branches, as prescribed by the By-Laws.

Article VII. Investment of Moneys and Funds. Funds of the ASSOCIATION, available for investment, may, with the approval of the Council, be invested by the Treasurer, in United States Government, State, Municipal, County or other securities acceptable as security for postal savings deposits, and in such securities only.

Article VIII. Amendments. Every proposition to alter or amend this Constitution shall be distributed to the members at least thirty days prior to the annual meeting, either by direct mail or in the JOURNAL. Every amendment shall be read at the first General Session of the annual meeting, and shall be balloted upon at a subsequent General Session

when, upon receiving the affirmative votes of two-thirds of the members present, it shall become a part of the Constitution. Any proposition to amend the Constitution for the purpose of permitting the expenditure of the permanent invested funds of the ASSOCIATION shall require a majority of seven-eighths of the members present for its passage.

BY-LAWS

(With all amendments to December 31, 1957)

CHAPTER I.—MEMBERSHIP

Article I. Kinds of Membership. In accordance with Article III of the Constitution, the Council may elect active, associate, life and honorary members of the ASSOCIATION.

Article II. Active Members. Any pharmacist of good professional standing is eligible to active membership. The Council may at its discretion grant membership to reputable nonpharmacists who are desirous of advancing the interests of pharmacy, and who are informed concerning the aims of the ASSOCIATION and willing to accept the obligations imposed by membership. The annual fee for active membership shall be determined by the Council.

Active members may vote at the annual sessions of the ASSOCIATION and participate in the election of the general officers.

Article III. Associate Members. Any student, undergraduate or graduate, regularly enrolled in any school or college of pharmacy, holding membership in the American Association of Colleges of Pharmacy or accredited by the American Council on Pharmaceutical Education, and having a duly organized Student Branch of this ASSOCIATION, may, upon application, be elected an associate member. The fee for such membership shall be set by the Council. Upon graduation, associate members shall automatically become active members, but no extra fee shall be charged for the remainder of the associate membership year. Graduate students, who devote at least one-half of their time to graduate study, may elect to continue as associate members until completion of graduate studies. Associate members shall not be entitled to vote nor to hold office, except in Student Branches.

Article IV. Life Members. Upon approval by the Council any person meeting the qualifications for active membership may be granted life membership under the following alternative conditions: (1) If he has been an active member and paid annual dues for forty consecutive years. (2) If he pays the life membership fee determined by the Council. Life members, who are exempt from all annual dues, shall continue to enjoy the privileges of active membership.

(In accordance with the foregoing By-Law the Council has passed the following rule: Any person who meets the qualifications for active membership in the ASSOCIATION and who shall pay to the Treasurer the sum of \$250, and also any active member not in arrears who after ten years shall pay the sum of \$200, or after fifteen years the sum of \$150, or after twenty years the sum of \$120, or after twenty-five years the sum of \$75, may become a life member.)

Upon the death or resignation of a life member, the life membership fee paid by such member shall be transferred from the life membership fund to the general fund of the ASSOCIATION.

Article V. Honorary Members. Professional and scientific workers of note and other persons of distinction who are recommended to and approved by the Council may be elected to honorary membership. They shall not be required to pay dues, nor shall they be eligible to hold office or to vote.

Article VI. Election. Membership Year. Each application for membership shall bear the endorsement of two active members of the ASSOCIATION in good standing and shall be accompanied by the dues for at least one year. A two-thirds vote of the Council is required for election to any class of membership.

The period of membership shall be one year from the first day of the month immediately following the date of election, and may be renewed for like periods by the further payment of the annual dues.

Each member shall pay in advance the annual dues of the class to which he belongs.

Article VII. Suspension. Reinstatement. All members whose annual dues are in arrears for a period of more than six months after payment is due shall be dropped from membership.

Any member whose membership has been withdrawn for nonpayment of dues may be readmitted by submitting the usual one year's membership fee and making application for membership as if he were a new member; or he may be readmitted without such application on payment of all dues in arrears. In the latter case his membership shall date from the time when he first joined the ASSOCIATION.

Article VIII. Resignations. Resignation of membership shall be made in writing to the Secretary, but no resignation shall be accepted from anyone in arrears to the treasury. The Secretary shall acknowledge all resignations in writing and shall report them to the Council.

Article IX. Expulsion. Any member may be expelled for improper professional conduct or for violation of the obligations of the Constitution and By-Laws or the Code of Ethics adopted by the Association. No person shall be expelled unless he shall have been given due notice of the charges and shall have had an opportunity to be heard by the Council, or, upon his request, by a Committee appointed by the House of Delegates. All charges must be made in writing to the Council by at least two active members in good standing. The Council shall take such steps as may be necessary and fair to the accused to establish the accuracy of the charges. No person shall be expelled unless he shall have received for expulsion two-thirds of all the votes cast at an executive session of the

House of Delegates.

Article X. Journals, Subscriptions. The subscription rates of the JOURNALS of the Association shall be fixed by the Council. Members of the Association shall be entitled to a reduction in the regular subscription rates of the JOURNALS and such subscriptions may be combined with or made a part of the annual dues fixed by the Council.

Publications of the Association which are issued on a periodic subscription basis shall be sent to each subscriber for the period covered by the subscription. In the case of members of the Association whose subscription fees are combined with the annual dues, the period during which the publications are supplied shall coincide with the membership year. The names of subscribers or members who are in arrears for two months after payment is due shall be dropped from the subscription list and the Association shall assume no obligation for supplying missing numbers of JOURNALS for the period in which the subscription or membership was in arrears.

CHAPTER II.—ELECTION OF OFFICERS

Article I. Nomination of President, Vice-President, Councilors, Chairman, and Vice-Chairman of the House of Delegates. Not less than three months prior to the month of the Annual Meeting, the Secretary of the Association shall send to each member of the House of Delegates a blank on which the member may nominate one candidate for each of the offices of President, First Vice-President, Second Vice-President, and Councilor, and Chairman and Vice-Chairman of the House of Delegates, all of whom shall be members of the AMERICAN PHARMACEUTICAL ASSOCIATION in good standing. The nominations shall be returned not less than two months prior to the opening of the Annual Meeting. The Secretary shall prepare an alphabetical list of eligible persons having received two or more nominations and submit this list to the Chairman of the Nominating Committee of the House of Delegates. This list shall be announced by the Secretary at the first session of the House of Delegates. At the first session additional nominations may be presented for an office, provided that such nominations shall be attested by two Delegates. From this combined list the Nominating Committee shall present to the second session for approval by the House of Delegates three candidates, one of whom shall be, as hereinafter provided, elected President to serve for one year, three candidates for First Vice-President, one of whom shall be elected for one year, three candidates for Second Vice-President, one of whom shall be elected for one year, and nine candidates for Councilor, three of whom shall be elected for three years, and one candidate for each of the offices of Chairman, and Vice-Chairman of the House of Delegates who shall be members of the House of Delegates. No person shall be nominated for the office of Councilor who shall have been elected to this office

for two consecutive terms unless one convention shall have been held between the end of his second term and the beginning of the term for which he is nominated. The Council is empowered and directed to fill all vacancies in the list of candidates, which may occur by death or resignation, after the adjournment of the Annual Meeting of the Association and prior to the issuance of mail ballots, and having due regard for the conditions of eligibility hereinabove set forth.

Article II. Election of Officers. The names of the candidates for Association officers shall be submitted by mail to every dues-paid, active member of the Association by the Secretary within two months after their nomination, together with a request that the member indicate on a ballot enclosed for that purpose his choice of candidates for the offices to be filled and return the same by mail within thirty days of the date printed on the ballot. The officers of the House of Delegates shall be elected as provided in the By-Laws of the House of Delegates.

Article III. Counting of Ballots. The ballots received within thirty days of the date printed on the ballots are to be delivered by the Secretary to the Board of Canvassers, who shall count the votes of active, dues-paid members only, and shall in turn certify to the Secretary the result of the election, after which the names of the successful candidates shall be published in the JOURNAL of the Association.

Article IV. Installation of Officers and Councilors. The officers thus elected by a plurality of votes, together with the other officers elected as hereinafter provided, shall be installed at the final general session of the first annual meeting of the Association following their election.

Article V. Election of Honorary President. The Honorary President of the ASSOCIATION shall be elected annually by the House of Delegates at its second session on nomination by the Council.

Article VI. Election of the Secretary and the Treasurer. The Secretary and the Treasurer of the ASSOCIATION shall be elected triennially by the House of Delegates at its second session on nomination by the Council.

CHAPTER III.—DUTIES OF THE OFFICERS AND THE COUNCIL

Article I. President. The President shall preside at all general sessions of the ASSOCIATION and shall perform the customary and parliamentary duties established by usage. He shall prepare an address to be presented at the first general session of the annual meeting following his installation. He shall nominate the members of the standing committees unless otherwise provided for, and submit the names of the nominees to the Council for ratification at the first meeting of the Council held after his installation. He shall be an *ex-officio* member of the Council.

Article II. Vice-Presidents. In the event of the decease or incapacity of the President, the First Vice-President shall assume the duties of the President for the unexpired term of office, and in the event of the decease or incapacity of both the President and First Vice-President, the Second Vice-President shall assume the duties of the President for the unexpired term of office.

Article III. Secretary. The Secretary shall keep the minutes of the general sessions of the ASSOCIATION and of the House of Delegates. He shall verify the credentials of the members of the House of Delegates. He shall prepare for publication the official program. He shall give notice of the time and place of annual and special meetings of the ASSOCIATION and of the House of Delegates. He shall notify members and officers of their election and committee members and delegates of their appointment. He shall revise the roll of members. He shall collect the dues of the members of the ASSOCIATION and remit the same to the Treasurer of the ASSOCIATION. He shall perform such other duties as may be directed by the House of Delegates or Council. He shall be an *ex-officio* member of the Council. The Secretary shall also serve as General Manager of the ASSOCIATION and shall have executive supervision over its activities, including direction of and responsibility for the headquarters building and the activities of the ASSOCIATION carried on therein subject to such limitations as are otherwise provided in these By-Laws or as may be provided for by action of the Council. In the event of the resignation, decease or incapacity of the Secretary, the vacancy shall be filled by vote of the Council until the next annual meeting of the ASSOCIATION.

Article IV. Treasurer. The Treasurer shall be the custodian of all moneys, securities and deeds belonging to the ASSOCIATION, and shall hold the same subject to the direction and disposition of the Council. He shall be an *ex-officio* member of the Council. In the event of the resignation, decease or in-

capacity of the Treasurer, the vacancy shall be filled by vote of the Council until the next annual meeting of the ASSOCIATION.

Article V. Bonding of Officers and Employees. The Secretary and Treasurer and such other officers and employees of the ASSOCIATION, as the Council may direct, shall be bonded for the proper care and disposition of the funds of the ASSOCIATION which may come into their hands, in such amounts and in such manner as may be prescribed by the Council.

Article VI. Membership of the Council and the Executive Committee of the Council. The Council shall consist of 16 members comprising nine elected Councilors, the President, the immediate Past President, the Vice-Presidents, the Secretary and the Treasurer of the ASSOCIATION, and the Chairman of the House of Delegates. The term of each member shall cease when his successor has qualified.

The President, the Chairman of the Council, the Chairman of the House of Delegates, the Secretary and the Treasurer shall constitute the Executive Committee of the Council.

Article VII. Vacancies. Vacancies among elected Councilors shall be filled by vote of the Council for the remainder of the unexpired term of the person replaced. In filling such vacancy the Council shall make its selection from the list of nominees at the last previous election.

Article VIII. Meetings—Quorum. The Council shall meet immediately after the Annual Meeting of the ASSOCIATION and at such other times as it may determine, or at the call of the Chairman, or on a call signed by a quorum of the Council, or as otherwise provided for. Nine members shall constitute a quorum.

Article IX. Duties. The Council shall have supervision of all property, funds, finances and publications of the ASSOCIATION. It shall select the Editors of the ASSOCIATION publications. It shall fix the date and select the hotel headquarters for the Annual Meeting, shall name the Local Secretary and may select a place for the Annual Meeting when, for any reason, the place chosen by the House of Delegates is found to be unavailable. It shall nominate, for election by the House of Delegates, the Honorary President, the Secretary and the Treasurer of the ASSOCIATION. It shall fix the dues of members and the subscription and sale prices and advertising rates of the publications of the ASSOCIATION. It shall fix the salaries and emoluments of officers and employees and shall prepare an annual budget for the ASSOCIATION. It shall ratify the standing committees nominated by the President unless otherwise

provided for. It shall act on applications for membership in the ASSOCIATION, and shall perform such other functions as may be designated in the By-Laws or be assigned from time to time by the ASSOCIATION and the House of Delegates. The Council shall act for the ASSOCIATION and for the House of Delegates in the interim between meetings and shall submit through its Secretary to the House of Delegates at its first session during the annual meeting an annual report

Article X. National Formulary and Supplements. The Council shall from time to time provide for the publication of such new editions of the National Formulary and for the publication of such supplements thereto as it may deem necessary. The Council shall also by resolution specify the dates from and after which such new editions of the National Formulary or of the Supplements thereto shall supersede the revision previously official.

CHAPTER IV.—HOUSE OF DELEGATES

Article I. Duties of the House of Delegates. It shall be the function of the House of Delegates to interpret the objectives of the AMERICAN PHARMACEUTICAL ASSOCIATION as stated in the Constitution in terms of contemporary requirements and to serve as the legislative and policy forming body of the ASSOCIATION.

(a) It shall be the duty of the House to give consideration to all appropriate proposals, emanating from constituent bodies represented in the House of Delegates.

(b) The officers of the House of Delegates are hereby charged with the duty of arranging the programs of interim and annual meetings of the House, so as to allow for a full discussion and debate of contemporary problems, thereby providing the basis for a long-range planning and the establishment of current and long-range policies.

(c) The officers of the House of Delegates in collaboration with the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION are further charged with the duty of properly delegating recommendations of the House to the appropriate officers and committees so as to assure dissemination of information on the policies adopted and their implementation by whatever means may be feasible and desirable

Article II. Membership. The House of Delegates shall be composed of accredited representatives of the following organizations representing the members of the ASSOCIATION, apportioned as stated below, and of certain *ex-officio* members as enumerated below. All members of the House of Delegates shall be duly qualified members of the AMERICAN PHARMACEUTICAL ASSOCIATION for not less than three consecutive years at the time of their appointment and shall maintain their membership during their term of service as Delegates.

(a) One delegate from each of the sections of the ASSOCIATION.

(b) One delegate from each local branch of the ASSOCIATION which is currently in good standing, provided that if the local branch has more than three hundred members, one delegate shall be elected for each two hundred members or major fraction thereof.

(c) One from each State Pharmaceutical Association and each territorial Pharmaceutical Association which has been officially recognized by the AMERICAN PHARMACEUTICAL ASSOCIATION for at least one year and one additional representative for each additional two hundred or major fraction of two hundred dues-paid members of the AMERICAN PHARMACEUTICAL ASSOCIATION where such membership of the State Association or territorial association exceeds two hundred.

(d) The members of the Council, the President-elect, and the Past Presidents shall be delegates ex-officio. The retiring chairman of the House of Delegates shall become a delegate ex-officio for a period of five years following the expiration of his term of office. Past chairmen of the House of Delegates who served as chairmen of the House of Delegates at the 1950, 1951, 1952, 1953 and 1954 conventions shall be ex-officio members of the House of Delegates until a five-year period following the convention at which they served as chairmen of the House of Delegates has expired.

(e) One delegate from each of the following named organizations: National Conference of State Pharmaceutical Association Secretaries, the Plant Science Seminar, American College of Apothecaries, American Society of Hospital Pharmacists, National Association of Retail Druggists, National Association of Boards of Pharmacy, American Association of Colleges of Pharmacy, National Wholesale Druggists' Association, Federal Wholesale Druggists' Association, American Drug Manufacturers' Association, American Pharmaceutical Manufacturers' Association, the Proprietary Association of America, National Association of Chain Drug Stores, American Institute of the History of Pharmacy and such other organizations as may be officially recognized by the AMERICAN PHARMACEUTICAL ASSOCIATION as entitled to representation.

(f) In the absence, for good and sufficient reason, of a fully qualified delegate from any organization entitled to membership in the House of Delegates and on recommendation of the Committee on Credentials and the unanimous consent of the House of Delegates, a duly authorized representative of any organization entitled to membership in the House of Delegates who may not meet, in full, the qualifications for membership in the House of Delegates may be seated as an alternate delegate.

Article III. Term of Service.

(a) In the initial formation of the House of Delegates under these By-Laws, one-third of the delegates shall be elected or appointed for terms of one year, one-third for terms of two years and one-third for terms of three years; the designation of the terms for which election or appointment shall be made, shall be determined by lot under the direction of the presiding officer prior to adjournment of the Annual Meeting at which this By-Law is adopted. The House shall adopt such additional rules as may be necessary in providing for the expiration of the terms of one-third of the membership each year. Thereafter the term of each elected or appointed delegate shall be for three years. These provisions shall not apply to delegates *ex-officio*.

(b) The Secretary of each constituent or affiliated organization entitled to elect or appoint one or more delegates shall certify to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION not later than January 1 of each year, the name, address and term of each elected or appointed delegate from said organization for the ensuing year and shall name the individual or individuals to be disqualified as a result of any officially determined change in the number of delegates to which said organization is entitled under the By-Laws.

(c) The term of office of the delegates *ex-officio*, shall be for the term of their service in their respective offices.

Article IV. Alternate Delegates.

(a) No alternate delegate shall be qualified for the delegates *ex-officio*.

(b) The secretary of each constituent or affiliated organization entitled to elect or appoint one or more alternate delegates shall certify to the Secretary, not later than 30 days before the date of the annual meeting each year, the name, address and term of each elected or appointed alternate delegate from said organization for the ensuing year.

(c) An alternate delegate may gain admission to the sessions of the House of Delegates upon the presentation of duly certified credentials from the organization from which he was elected or appointed. He may not vote on any matter coming before the House of Delegates except on due notice by the Secretary that the accredited delegate he represents is absent for good and sufficient reasons.

Article V. Meetings—Quorum.

(a) The House of Delegates shall meet not later than the second day of the annual meeting of the ASSOCIATION and thereafter as necessary to transact the business of the House of Delegates or upon the

call of the Chairman, or upon the written request of one-third of the qualified delegates representing a majority of the affiliated State Associations, provided that the House of Delegates shall conclude its business prior to the final general session of the ASSOCIATION; provided further that the House of Delegates shall hold a meeting of not less than two days during the interim between the conventions of the Association, and the Chairman and Secretary shall be vested with authority to arrange for the time and place of such interim meetings. Insofar as practical, the interim meetings shall be held either in October or November following the annual convention of the Association.

(b) Special meetings of the House of Delegates may be called in the interim between annual meetings of the ASSOCIATION at the call of the Chairman with the consent of the Council or by a resolution passed by a majority of the Council, in either case with the written assent of one-third of the voting delegates, or upon written request of one-third of the voting delegates.

(c) A quorum for the transaction of business shall be declared if one-third of the total number of qualified delegates constituting the House of Delegates shall be present at any regular or special meeting of the House of Delegates.

(d) The House of Delegates shall adopt By-Laws for the conduct of its business which shall not be inconsistent with the Constitution and By-Laws of the ASSOCIATION.

Article VI. Officers. The officers shall consist of a Chairman and a Vice-Chairman. The Secretary of the ASSOCIATION shall act as Secretary of the House of Delegates. These officers shall serve for one year or until their successors are installed. The Chairman shall present an address, at the first session of the annual meeting of the House, upon any subject which he deems of pharmaceutical importance.

Article VII. Selection of Time and Place of Annual Meeting and General Business. The House of Delegates shall select the time and place of the annual meeting and shall transact all general business of the ASSOCIATION not otherwise provided for in the Constitution and By-Laws.

Article VIII. Fraternal Delegates. Fraternal delegates may be received from any of the departments of the United States Government, from the student branches of the AMERICAN PHARMACEUTICAL ASSOCIATION, and such other organizations as the House of Delegates may determine. Such fraternal delegates may be granted the privilege of the floor but shall not be entitled to vote.

CHAPTER V.—SECTIONS

Article I. Sections and Additions. To expedite and render more efficient the work of the ASSOCIATION, the following sections are provided:

1. Scientific Section.
2. Section on Education and Legislation.
3. Section on Practical Pharmacy.
4. Section on Pharmaceutical Economics.
5. Section on Historical Pharmacy.

Additions to or deletions from the list of sections may be made by the House of Delegates upon the recommendation of the Council.

Article II. Membership. Attendance upon the meetings of the sections and the presentation of competent papers therein shall be open to any member of the AMERICAN PHARMACEUTICAL ASSOCIATION. Each section, with the approval of the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, shall establish the qualifications for membership in such section and no person shall be eligible to vote or hold office in such section who does not satisfy the requirements for membership.

Article III. Meetings—Quorum. The sections shall meet at such time and place, during the annual meeting of the ASSOCIATION, as may be provided for in the annual program. Fifteen members shall constitute a quorum at any meeting of a section.

Article IV. Delegate to the House of Delegates. Each section shall elect by ballot a voting delegate to the House of Delegates who shall hold office for the period of time for which he is elected or until a successor is elected.

Article V. Officers. Each section shall, with the approval of the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, adopt such By-Laws, rules and regulations, and name such officers and com-

mittees as may be necessary for the proper conduct of its work.

Article VI. Duties—Records. Each section through its officers shall solicit papers and propose suitable subjects for discussion at its annual meetings, arrange the business of the section in advance and perform such duties as may be referred to it. It shall make an annual report to the House of Delegates. The minutes of each meeting, together with all documents and papers which belong to each section, must be placed immediately after the meeting in the hands of the Secretary of the ASSOCIATION for publication and safe-keeping.

Article VII. Papers—Submission and Disposal. Any person desiring to submit a paper to the ASSOCIATION shall present to the secretary of the section to which it refers, at least 10 days prior to the annual meeting, an abstract of said paper indicating its contents and consisting of not less than 50 nor more than 200 words. The paper itself shall be submitted to the officers of the section for approval prior to its first meeting. Not more than 15 minutes shall be allowed for the presentation of any paper unless by the unanimous consent of the section. All papers presented to the sections shall become the property of the ASSOCIATION with the understanding that they are not to be published in any other publication prior to their publication in those of the AMERICAN PHARMACEUTICAL ASSOCIATION, except with the consent of the editor of the appropriate JOURNAL and the Chairman of the Committee on Publications of the Council. Papers and addresses submitted by special invitations of the officers of the sections shall not be subject to the requirements of this Article.

CHAPTER VI.—LOCAL BRANCHES AND STUDENT BRANCHES

Article I. Local Branches of the American Pharmaceutical Association.

(a) A local branch of the AMERICAN PHARMACEUTICAL ASSOCIATION may be established by the Council upon the petition of not less than 15 members of the ASSOCIATION, in good standing residing within the area defined in their petition.

(b) A local branch shall be chartered on the basis of whole counties at least some part of which does not lie within a radius of 45 miles from the headquarters of the local branch. However, when counties exceed 10,000 square miles in area, or where the topographical nature of the county makes it seem desirable, the Council may, at its discretion, permit the county to be divided among two or more local branches. Both headquarters of the local branch and territory are subject to the approval of the Council. Changes in headquarters or territory of a local branch already chartered shall be made only by vote of the Council.

(c) Any local branch chartered in accordance with the provisions of this By-Law which fails to maintain a paid membership in the ASSOCIATION of 15 or more members, as provided in the By-Laws, shall automatically forfeit its charter.

(d) A local branch may be dissolved by the Council for good and sufficient reasons.

(e) All members of the AMERICAN PHARMACEUTICAL ASSOCIATION in good standing, residing in that portion of the United States chartered by the Council as the territory of a local branch shall be considered members of that local branch and shall be so enrolled and shall be entitled to all privileges such local branch may grant under the Constitution and By-Laws of the ASSOCIATION, provided, however that members not residing within the territory of a local branch may be enrolled therein as prescribed in the By-Laws.

(f) Each local branch shall make By-Laws for its own government which shall be subject to the approval of the Council and not inconsistent with the Constitution and By-Laws of the ASSOCIATION. Such By-Laws shall be submitted to the Council, through the Executive Secretary of the ASSOCIATION for approval.

(g) A local branch shall elect its own officers and delegates to the House of Delegates. All selections for office and changes in office shall be officially transmitted promptly by the Secretary of the local branch to the Executive Secretary of the ASSOCIATION.

(h) No local branch or committee thereof or local branch officer, acting in an official capacity, shall issue any resolution or statement or take official action in the name of the AMERICAN PHARMACEU-

TICAL ASSOCIATION without approval of the Council of the ASSOCIATION.

(i) A local branch shall receive a refund of a portion of the annual dues paid by its members to the ASSOCIATION as provided by the Council.

(j) A local branch may assess dues and raise or collect funds to be expended for local purposes in harmony with the provisions of the Charter of the ASSOCIATION and may have the entire management and control of these funds.

(k) A local branch may receive donations or bequests made to said local branch and may expend or invest the same in the interest of said local branch at the discretion of its governing body.

(l) Each local branch shall submit to the Council an annual report of its operations including an itemized statement of receipts and expenditures and investments of its funds.

Article II. The Student Branches of the American Pharmaceutical Association.

(a) A Student Branch of the AMERICAN PHARMACEUTICAL ASSOCIATION may be established by the Council upon the petition of not less than 15 students in good standing, matriculated in a College, School, Department or Division of Pharmacy holding membership in the American Association of Colleges of Pharmacy and/or accredited by the American Council on Pharmaceutical Education. The petition of such students shall be recommended in writing by not less than five members of the ASSOCIATION, three of which shall be members of the Faculty of the College, School, Department or Division of Pharmacy in which the students are enrolled.

(b) Members of a Student Branch shall pay an annual membership fee to the ASSOCIATION in an amount fixed by the Council, a portion of said annual membership fee shall be credited to a subscription to the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, *Practical Pharmacy Edition*, and a second portion, to be determined by the Council, shall be returned to the Student Branch to be expended by the said Student Branch in the furtherance of the aims and objectives of the ASSOCIATION.

(c) Members of a Student Branch shall be known as Junior Members of the AMERICAN PHARMACEUTICAL ASSOCIATION until they have completed the requirements for the degree in Pharmacy.

(d) Any Student Branch which fails to maintain a dues-paid membership of not less than 15 regularly matriculated students shall automatically forfeit its charter.

(e) Any Student Branch may be dissolved by the Council for good and sufficient reasons.

CHAPTER VII.—AFFILIATED ORGANIZATIONS

Organizations entitled to representation in the House of Delegates, which carry on activities designed to encourage and promote public health services and the advancement of pharmacy in this field, may be designated as affiliated organizations and their individual members may be designated as affiliates.

To be designated as an affiliated organization, the Executive Committee or other governing body of the organization shall apply to the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION for such designation. If the Council approves such designation, it shall fix the terms of affiliation by means of a

written agreement mutually satisfactory and signed by the officers of the respective organizations. Such agreement shall include, among other provisions, reference to the extent of the services to be supplied by the staff of the AMERICAN PHARMACEUTICAL ASSOCIATION and its publications; the annual dues or other financial contribution of the affiliated organization, if any; the limitations of joint public statements; and the use of the name of the AMERICAN PHARMACEUTICAL ASSOCIATION in any joint or individual pronouncements. Affiliation may be terminated by the Council on due notice for cause or upon mutual consent of the parties to the agreement.

CHAPTER VIII.—FINANCES

Article I. Source of Funds. The necessary funds for carrying on the activities of the ASSOCIATION may be derived from membership dues, from the publications of the ASSOCIATION, from sustaining funds, maintenance funds, grants, bequests, contributions, investments and from any other sources approved by the Council. The Council shall appropriate the funds necessary to defray the expenses of the ASSOCIATION.

Article II. Deposit of Moneys. The Treasurer shall deposit all moneys received by him with a reliable banking company, or companies, recommended by the Committee on Property and Funds and approved by the Council, where said moneys may be drawing interest for the benefit of the ASSOCIATION, unless otherwise provided for. Said moneys shall be deposited in the name of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article III. Payment of Moneys or Funds. Moneys or funds shall be paid out by numbered voucher checks signed by the Secretary and Treasurer.

Article IV. Payment of Bills. Unless otherwise provided for, the correctness of every bill shall be certified by the person contracting the same and by the Secretary, and the latter shall note on the bill the appropriation against which the bill is to be charged. A voucher check showing the appropriation against which the payment is charged shall then be drawn and signed by the Secretary. Upon receipt of the check, together with the original bill, the Treasurer shall complete the check, affix his signature and return the check with the bill to the Secretary who shall forward the check and file the bill. In the event of the incapacity of either the Secretary or the Treasurer, the signature of the Chairman of the Council shall be accepted in lieu of that of the officer incapacitated.

Article V. Investment of Money or Funds Unless otherwise provided for, the money or the funds

of the ASSOCIATION may be invested in bonds, deeds and other securities upon recommendation of the Committee on Property and Funds and the approval of the Council.

Article VI. Designation of Banks and Safe Deposit Vaults for Funds, Securities and Deeds. The Committee on Property and Funds shall annually recommend to the Council for approval the banks and safe deposit vaults in which the funds, securities and deeds of the ASSOCIATION shall be kept for the ensuing year.

Article VII. Custodian of Funds, Securities, and Deeds. The bank books, securities and deeds shall be in the name of the AMERICAN PHARMACEUTICAL ASSOCIATION with the Treasurer as custodian, and the accounts of the same shall be kept by him.

Article VIII. Auditing of Accounts. The Committee on Finance shall on or about December 1 of each year recommend to the Council for its approval a certified public accountant, or accountants, who shall audit all accounts of the ASSOCIATION before March 1 of the next fiscal year, and who shall report the results of the audit promptly to the Council through its Secretary.

Article IX. Annual Reports of the Secretary and Treasurer. The Secretary and Treasurer shall balance the accounts of the ASSOCIATION and prepare their financial reports as promptly as possible after the close of the fiscal year. Their reports, with the books, accounts, vouchers and other pertinent data, shall be promptly made available to the accountant or accountants for audit.

Article X. Expense of Bonds of Secretary and Treasurer. The expense of the bonds required to be given by these officers or any other officers or employees of the ASSOCIATION shall be paid by the ASSOCIATION.

Article XI. Merging of Balances. All balances

CHAPTER VIII.—FINANCES (Continued)

remaining from appropriations at the close of each fiscal year shall be turned back into the treasury unless otherwise ordered by the Council.

Article XII. Fiscal Year. The fiscal year of the AMERICAN PHARMACEUTICAL ASSOCIATION shall coincide with the calendar year.

Article XIII. Expenditure of Funds. Any action taken by the ASSOCIATION, or by the House of Delegates, or by any of the Sections, which involves an expenditure of funds of the ASSOCIATION, shall be submitted to the Council for consideration and final disposition.

CHAPTER IX.—STANDING AND SPECIAL COMMITTEES

Article I. Standing Committees. Unless otherwise provided for, there shall be nominated by the President and ratified by the Council the following Standing Committees:

1. Committee on Local Branches.
2. Board of Canvassers.
3. Committee on Membership.
4. Committee on Legislation.
5. Committee on United States Pharmacopeia.
6. Committee on National Formulary.
7. Committee on Pharmaceutical Research.
8. Committee on Ebert Prize.
9. Committee on Kilmer Prize.
10. Committee on Public Relations.
11. Committee on the Status of Pharmacists in Government Service.
12. Committee on Policy and Planning.
13. Committee on Student Branches.
14. Committee on Social and Economic Relations.
15. Committee on Professional Relations.
16. Committee on Constitution and By-Laws.
17. Committee on National Defense and Security.
18. Committee on International Relations.

Article II. Board of Canvassers. The Board of Canvassers shall be composed of three members appointed by the President. It shall carry out the duties provided for under the election of officers.

Article III. Committee on Membership. The Committee on Membership shall be composed of a General Chairman, District Chairmen and State Chairmen, the latter each with one or more associates. It shall be the duty of this committee to invite the membership of pharmacists and others interested in pharmacy and to cooperate with the state associations in the effort to bring their membership into the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article IV. Committee on U. S. P. The Committee on the United States Pharmacopeia shall be nominated by the President of the ASSOCIATION as follows: Two members to be appointed for five years, and two for four, three, two, and one years, respectively, each vacancy occurring by expiration of term to be filled by a new appointment for five years. The Committee shall elect its own chairman annually. It shall collect statistics regarding the frequency with which official and nonofficial remedies are used in legitimate practice, and shall endeavor to ascertain the general wishes and requirements of

the profession throughout the country in regard to any desired changes or improvements in the Pharmacopeia. It shall also note errors of any kind found in the U. S. Pharmacopeia so as to facilitate and aid the work of the National Committee of Revision of the U. S. P.

Article V. Committee on National Formulary. The Committee on National Formulary shall consist of a chairman elected by the Council for a term of five years and ten members elected by the Council two to serve for a term of one, two, three, four and five years, respectively, and the Director of the A. P. H. A. laboratory who shall be a member of the Committee, *ex-officio*; each vacancy occurring from expiration of term shall be filled by election for the unexpired term. The Committee shall elect a Vice-Chairman and a Secretary from its own membership. This Committee shall serve as an executive committee of revision of the National Formulary. The chairman of the Committee on National Formulary, with the advice and consent of the members of the executive committee shall nominate to the Council additional participating members to the number of not more than 50, to constitute an advisory panel on National Formulary. Sub-committees may be organized for general or specific projects as required by the executive committee. The Committee on National Formulary shall report annually, or as often as required, to the Council.

Article VI. Committee on Pharmaceutical Research. The Committee on Pharmaceutical Research shall be elected by the Council, two members to serve for a term of five years, two for a term of four years, two for a term of three years, two for a term of two years, two for a term of one year and after the expiration of the one-year term two members shall be elected annually for a term of five years. The Chairman of the U. S. P. Revision Committee, the Chairman of the Committee on N. F., the Chairman of the Scientific Section of the A. P. H. A., the Secretary of the A. P. H. A. and the Treasurer of the A. P. H. A. shall be associate members of the Committee. The Committee on Pharmaceutical Research shall endeavor to promote research along pharmaceutical lines and shall advise the Council as to the use of the research funds of the ASSOCIATION.

Article VII. Committee on Ebert Prize. The Committee on Ebert Prize, which shall be appointed by the Chairman of the Scientific Section, with the approval of the Council, shall, at the next annual

meeting after the one at which papers are presented, determine which, if any of them, has met the requirements of the founder of the prize. In all respects it shall be governed by the stipulations expressed by the donor.

Article VIII. Committee on Kilmer Prize. The Committee on Kilmer Prize shall be appointed by the Chairman of the Scientific Section, with the approval of the Council, and shall award the Kilmer Prize in accordance with the rules and regulations which may from time to time be adopted by the Scientific Section and approved by the Council. In all respects it shall be governed by the stipulations expressed by the donor.

Article IX. Committee on Policy and Planning. The Committee on Policy and Planning shall be appointed by the President of the ASSOCIATION. It shall be the duty of this Committee to formulate a long-time program of policy by which the ASSOCIA-

TION may work steadily from year to year in an orderly fashion toward the attainment of the objectives as set forth in Article II of the Constitution. The Committee shall also discharge such other duties as may be assigned to it by the Council.

Article X. Duties of Standing Committees. The duties of the Standing Committees, unless otherwise provided for, shall be those assigned to them by the Council.

Article XI. Special Committees. Special Committees of the ASSOCIATION shall be appointed by the President of the ASSOCIATION, and unless otherwise provided shall consist of four members and a Chairman. Special Committees of the House of Delegates shall be appointed by the Chairman of the House of Delegates. Special Committees shall serve for a period of one year unless otherwise specified in the terms of their appointments.

CHAPTER X.—GENERAL MEETINGS

Article I. Time—Sessions. The meetings shall be held annually. In case a national emergency prevents holding a meeting as scheduled, it shall be the duty of the President with the advice and consent of the Council to call the next annual meeting as soon as practicable after the emergency. At least three general sessions of the annual meeting shall be provided for, the First General Session to be held on the first day of the annual meeting.

Article II. Order of Business. At the First General Session of the annual meeting, the President's address shall be received and referred to the House of

Delegates. At the Second General Session the address of the President-Elect shall be received. At the Final General Session the report of the House of Delegates shall be received and the officers for the ensuing year shall be installed. At any general session such other general business as may be presented may be transacted.

Article III. Authority of the General Sessions. Proceedings and official actions of the House of Delegates are subject to review and approval of the General Sessions.

CHAPTER XI.—RULES OF ORDER AND DEBATE

Article I. Rules and Appeals. The ordinary rules of parliamentary bodies shall be enforced by the presiding officer, from whose decision, however, appeals may be taken, if required by two members, and the meeting shall thereupon decide without debate.

Article II. Motions. When a question is regularly before the assembly and under discussion, no motion shall be received but to adjourn, to lay on the table, for the previous question, to postpone to a certain day, to commit or amend, to postpone indefinitely; which several motions have precedence in the order named. A motion to adjourn shall be decided without debate.

Article III. Debate. No member may speak

twice on the same subject, except by permission, until every member wishing to speak has spoken.

Article IV. Voting. On the call of any two members, the ayes and nays shall be ordered, when every member shall vote, unless excused by a majority of those present, and the names and manner of voting shall be entered on the minutes.

Article V. Points of Order. On all points of order not covered in these By-Laws, the ASSOCIATION shall be governed by the latest edition of Robert's Rules of Order.

Article VI. Quorum. Twenty-five active members shall constitute a quorum for the transaction of business.

CHAPTER XII.—MISCELLANEOUS

Article I. Quorum of Committees. A majority of the members of any of the standing or special committees shall constitute a quorum for the transaction of business.

Article II. Council and Committees—Voting by Mail. In all questions arising before the Council or before any of the Standing or Special Committees, and which can be disposed of by positive or negative

vote, the Chairman of the Council or the Chairman of any of these Committees may take the vote of their respective bodies in writing, and the same shall have the same force and effect as if members had been personally present, a majority of the votes cast being considered sufficient to decide a question. The ayes and nays of such votes taken by the Council shall be entered upon the minutes of said Council.

CHAPTER XII.—MISCELLANEOUS (Continued)

Article III. Conflict or Inconsistency. If any By-Law of the Council or House of Delegates or any of the Sections is found to be in conflict or inconsistent with these By-Laws, the provisions of these By-Laws

shall be given precedence until such time as the conflict or inconsistency may be corrected by proper amendment of the respective By-Laws.

CHAPTER XIII.—AMENDMENTS

Article I. Amendments. Every proposition to alter or amend these By-Laws shall be submitted in writing at a general session, referred to the Committee on Constitution and By-Laws for report, and

may be balloted upon at any subsequent general session, when, upon receiving the votes of three-fourths of the members present and voting, it shall become a part of the By-Laws.

BY-LAWS OF THE COUNCIL

(With all amendments to December 31, 1957)

CHAPTER I.—ELECTION AND DUTIES OF OFFICERS

Article I. Officers—Organization. The officers of the Council shall consist of a Chairman, a Vice-Chairman and a Secretary. The Secretary of the ASSOCIATION shall be the Secretary of the Council. No member of the Council shall hold the office of Chairman for more than two successive terms.

The Chairman and Vice-Chairman shall be elected and assume the duties of their respective offices at the organization meeting of the Council, which shall be held within twenty-four hours of the adjournment of the final general session of the annual convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article II. Duties of the Chairman. The Chairman shall preside at all meetings of the Council, and at his request, or in his absence, or on account of any inability to preside, the Vice-Chairman shall assume the duties of the Chairman, or, in the absence of both, a Chairman *pro-tempore* chosen by the Council shall perform the duties of Chairman.

Article III. Duties of the Secretary. The Secretary shall keep fair and correct minutes of the proceedings of the Council and shall carefully preserve all reports and communications received by the Council.

The Secretary shall call to order the organization meeting of the Council and preside during the election of its chairman. He shall likewise call for the election of a Chairman *pro-tempore* on the occasion of the absence or disability of the Chairman and Vice-Chairman.

The Secretary shall conduct the official correspondence of the Council and arrange for the transaction of the business of the Council by correspondence in the interim between meetings. He shall announce all votes promptly and keep the members of the Council informed of all business at regular intervals.

CHAPTER II.—COMMITTEES

Article I. Standing Committees. The Standing Committees of the Council shall consist of an Executive Committee, a Committee on Finance, a Committee on Property and Funds, a Committee on Publications, and an Advisory Committee on A. PH. A. Laboratory. The Committees on Finance, Property and Funds, Publications, and the Executive Committee shall be appointed entirely from the personnel of the Council.

Article II. Executive Committee. The Executive Committee shall consist of the President of the ASSOCIATION, the Chairman of the Council, the Chairman of the House of Delegates, the Secretary and the Treasurer of the ASSOCIATION. This Committee shall act for the Council in emergencies and shall discharge such other duties as may be assigned to it. All actions by the Executive Committee shall be reported to the Council.

Article III. Committee on Finance. The Committee on Finance shall consist of three members of the Council appointed by the Chairman with the

consent of the Council, and shall each year, prior to January 1, present to the Council for its consideration a tentative budget for the ensuing fiscal year. No payment shall be made in excess of any of the said appropriations of the budget finally approved by the Council for the fiscal year except by a special vote of the Council, provided, however, that the Treasurer is authorized to transfer from one appropriation account to another such amount as may be needed at any time, the amount of any such transfer not to exceed the sum of fifty (\$50.00) dollars.

All motions and resolutions involving the expenditure of any sum in excess of twenty-five (\$25.00) dollars shall have the approval of the Finance Committee before being voted upon by the Council.

All appropriations made for any fiscal year shall lapse at the end of the said fiscal year; provided, however, that accounts properly chargeable against any of said appropriations prior to their expiration, but not received by the Secretary of the ASSOCIATION until after the end of the fiscal year, may be paid

from such appropriation in case the warrant for such payment can be drawn not later than thirty (30) days after the close of said fiscal year.

Article IV. Committee on Property and Funds. The Committee on Property and Funds shall consist of the President, the Treasurer, the Chairman of the Council, the Chairman of the Committee on Finance and the Secretary of the ASSOCIATION.

It shall have charge of the administration of all of the property and established funds of the ASSOCIATION and shall carry out such duties as assigned to it in the By-Laws of the ASSOCIATION.

The Committee shall consider applications for grants from the income derived from the established funds and shall promptly report to the Council such recommendations as may be deemed proper. If and when the Council approves of any recommendation of the Committee, orders shall be drawn upon the Treasury in favor of those to whom grants have been made.

Article V. Committee on Publications. The Committee on Publications shall consist of the Secretary, the Treasurer and three other members to be appointed by the Chairman with the consent of the Council.

The Committee on Publications shall act in an advisory capacity in the editing, publication and distribution of the JOURNALS of the ASSOCIATION, the National Formulary and such other publications as may be issued under the rules and regulations to be approved by the Council. The Editors of the JOURNALS shall be elected by the Council.

Article VI. Advisory Committee on A. Ph. A. Laboratory. The Committee on A. Ph. A. Laboratory shall consist of five members of the ASSOCIATION elected by the Council. Each member of the Committee shall serve for a term of five (5) years, one member being elected each year. The Chairman of the National Formulary Committee, the Director of the A. Ph. A. Laboratory and the Director of Revision of the United States Pharmacopeia, and the Secretary of the ASSOCIATION shall be members *ex-officio* of the Committee. The Committee shall choose its own Chairman and Secretary.

The duties of this Committee shall be to give advice on the scope, policy and functions of the laboratory and the selection of personnel as requested by the Council.

Article VII. Committee on National Formulary. The Committee on National Formulary shall consist of a Chairman and ten (10) members to be elected by the Council. The Chairman of the Committee shall be elected for a period of five years. Two members shall be elected each year to fill the vacancies occurring that year and shall serve for a period of five years from the time of their election. The Committee shall annually elect a Vice-Chairman and a Secretary from among its own membership. The Chairman may be designated as the Director of Revision of the National Formulary.

Article VIII. Special Committees. Special Committees of the Council may be appointed, as needed. Special Committees shall serve only until the end of the annual meeting unless specific provision is made for their continuance.

CHAPTER III.—MEETINGS

Article I. Time and Quorum. In addition to the organization meeting, the Council shall meet prior to the assembling of the ASSOCIATION and at such other times as it may determine, or at the call of the

Chairman, or upon the application, in writing, of five elected members of the Council.

Nine members of the Council shall constitute a quorum.

CHAPTER IV.—ORDER OF BUSINESS

Article I. The order of business at regular meetings of the Council shall be as follows unless otherwise determined:

1. Call to order and roll call.
2. Election of officers (at organization meeting only).
3. Reading and approval of minutes.
4. Reading of Communications.
5. Reports of the Officers.

6. Reports of the Committees.
7. Unfinished and deferred business, such business as is specifically referred to the Council by the ASSOCIATION and business that may be specifically mentioned in the call for the Council meeting.
8. New business.
9. Election of members of the ASSOCIATION.
10. Adjournment.

CHAPTER V.—MISCELLANEOUS

Article I. Quorum of Committees. A majority of the members of any committee of the Council shall constitute a quorum for the transaction of business.

Article II. Motions—Voting by Mail. Questions arising in the interim between meetings of the Council or committees may be submitted to the members by mail in Council or Committee Letters, and motions so submitted do not require a second. A majority of the votes cast by mail, upon any proposition so submitted, provided the votes so cast repre-

sent a majority of the members of the Council or committee, shall be considered as the action of the Council or committee.

BY-LAWS OF THE COUNCIL (*Continued*)

sent a quorum of the Council or Committee, shall decide the question. Members of the ASSOCIATION may be elected by mail vote.

Article III. Amendments. Every proposition to alter or amend these By-Laws shall be submitted in

writing at one session and may be balloted upon at the next session of the Council, when, upon receiving a favorable vote of three-fourths of the members present, it shall become a part of these By-Laws.

BY-LAWS OF THE HOUSE OF DELEGATES

(With all amendments to December 31, 1957)

CHAPTER I

Article I. Opening of the House of Delegates. The First Session of the House of Delegates at each Annual Meeting shall be called to order by the Chairman, the Vice-Chairman or the Secretary.

Article II. Delegates. Immediately after the First Session of the House of Delegates has been called to order, the Secretary shall report the accredited representatives, who shall then compose the House of Delegates.

Article III. Voting. Each delegate shall be entitled to one vote. No delegate shall act as proxy of another delegate nor as delegate for more than one association or organization. An alternate dele-

gate shall function according to the provisions of Chapter IV, Article III, of the By-Laws of the ASSOCIATION.

Article IV. Fraternal Delegates. Fraternal delegates may be received from organizations not entitled to voting delegates. These delegates shall have the privileges of the floor in other than executive sessions but without vote.

Article V. Privileges. Any member of the AMERICAN PHARMACEUTICAL ASSOCIATION may attend any session of the House of Delegates other than an executive session, and shall have the privilege of the floor.

CHAPTER II

Article I. Officers of the House. The officers of the House of Delegates shall be a Chairman, a Vice-Chairman and a Secretary. The Secretary of the ASSOCIATION shall be Secretary of the House. The Chairman and Vice-Chairman shall be elected annually at the second session of the House subsequent to the report of the Committee on Nominations and shall serve for one year or until their successors shall be installed.

The Chairman and Vice-Chairman shall be installed at the final session of the House held during the annual meeting of the ASSOCIATION.

In the event of the resignation, decease or in-

capacity of either the Secretary or the Treasurer of the ASSOCIATION, the vacancy shall be filled by the Council until the next meeting of the House of Delegates whereupon the House of Delegates shall receive and act upon the nomination made by the Council.

Article II. Election of the Honorary President, the Secretary and Treasurer of the Association. At the Second Session of the House of Delegates held during the annual meeting of the ASSOCIATION, the House shall elect the Honorary President and triennially the Secretary and Treasurer of the ASSOCIATION, all on nomination of the Council.

CHAPTER III

Article I. Duties of the Chairman and Vice-Chairman. The Chairman shall preside at all meetings of the House of Delegates, except in the case of his absence or on account of inability to preside from any other causes, when the Vice-Chairman shall preside. In the event of the inability of both the Chairman and Vice-Chairman to preside, a temporary Chairman shall be elected by the House of Delegates, except at the opening of the First Session of the House when the Secretary shall preside as hereinbefore provided. The presiding Chairman shall perform the duties specified or referred to in

Chapter IV of the By-Laws of the ASSOCIATION and the customary and parliamentary duties established by usage.

The Chairman shall prepare, with the assistance of the Secretary, the annual report of the House of Delegates.

Article II. Duties of the Secretary. The Secretary shall perform the duties specified in the By-Laws of the House and of the ASSOCIATION or otherwise assigned by the House. He shall read all communications and papers received for that pur-

pose, shall call and record the ayes and nays whenever they are required to be called, he shall notify committees of the House of Delegates of their appointment, stating the business on which respective committees are to act. With respect to the verification of credentials of the members of the House of Delegates, the Secretary shall, at least two months before the annual meeting, send appropriate blank credentials for delegates to the various bodies entitled to representation in the House of Delegates and notify the said bodies of the time when the credentials properly filled out shall be returned to him. He shall report the verified cre-

dentials to the First Session of the House of Delegates as hereinafore provided. Credentials received and verified after the First Session of the House of Delegates shall be reported at the Second Session. The proceedings of the House of Delegates as submitted by the Secretary shall be published annually and the Secretary shall procure a sufficient number of copies of the same for distribution among the members of the House of Delegates and the officers of the ASSOCIATION and on request to members of the ASSOCIATION. Said proceedings shall also contain a list of the members, officers and committees of the House of Delegates.

CHAPTER IV

Article I. Committee on Nominations. At its First Session held during the annual meeting of the ASSOCIATION, the Chairman shall appoint a Committee on Nominations consisting of nine members whose duty it shall be to nominate the candidates for the office of President, First Vice-President, Second Vice President, the candidates for membership in the Council, and Chairman and Vice Chairman of the House of Delegates as provided in Chapter II of the By Laws of the ASSOCIATION. The report of the Committee on Nominations shall be received and acted upon at the Second Session of the House of Delegates as directed in Chapter II of the By Laws of the ASSOCIATION.

Article II. Committee on Place of Meeting. The Chairman of the House of Delegates shall within sixty days after his installation appoint a Committee on Place of Meeting consisting of five members whose duty it shall be to consider invitations. The report of this Committee shall be received and acted upon at the Second Session of the next annual meeting of the House of Delegates.

Article III. Committee on Resolutions. The Committee on Resolutions shall consist of nine members appointed by the Chairman at the First

Session upon the adoption of these By Laws, to which shall be referred the address of the President of the ASSOCIATION, the address of the Chairman of the House of Delegates and such resolutions and reports as are submitted or referred to the House of Delegates. The Chairman shall appoint nine members, three of whose terms shall expire in one, two and three years respectively. Thereafter, three are to be appointed annually. The President of the ASSOCIATION and the Chairman of the House of Delegates shall be *ex officio* members. The Chairman of the House shall name the Chairman of the Committee each year and the Secretary of the ASSOCIATION shall be Secretary of the Committee.

The Committee may report to the House at any of the sessions and shall make its final report at the final session of the House.

Article IV. Special Committees. The Chairman shall appoint such special committees of the House of Delegates as may be necessary. (Unless otherwise provided, Committees shall consist of a chairman and four members.) Special committees shall expire at the end of the next annual meeting unless specific provision is made for their continuance.

CHAPTER V

Article I. Motions and Resolutions. All motions and resolutions shall receive for adoption the affirmative vote of the majority of the members present and voting, such action to be final unless revoked by three fourths of the ASSOCIATION'S Membership present and voting.

Article II. Amendments. Every proposition to

amend the By Laws of the House of Delegates shall be submitted in writing at one session of the House and may be acted upon at the next session, when, upon receiving the affirmative vote of three fourths of the members present, it shall become a part of these By Laws.

CHAPTER VI

Article I. Order of Business. The following shall be the order of business unless otherwise determined:

- 1 Call to order
- 2 Roll call of delegates
- 3 Reading and adopting of the minutes
- 4 Appointment of committees
- 5 Address of the Chairman of the House of Delegates
- 6 Receipt of reports and other communications from the ASSOCIATION, the Council and Sections, all of which shall be in writing

- 7 Receipt of resolutions, reports and other communications, all of which shall be in writing
- 8 Unfinished business
- 9 New business
- 10 Nomination of officers of the ASSOCIATION to be elected by mail ballot, election of officers of the ASSOCIATION upon nomination by the Council and election of officers of the House
- 11 Installation of officers of the House
- 12 Adjournment

BY-LAWS OF THE HOUSE OF DELEGATES (*Continued*)

CHAPTER VII

Article I. Rules of Order. The procedure of the House of Delegates shall be governed by the latest edition of Robert's Rules of Order when not

in conflict with these By-Laws or with any rules adopted by the House.

BY-LAWS OF THE SCIENTIFIC SECTION

(With all amendments to December 31, 1957)

CHAPTER I.—NAME AND OBJECTS

Article 1. Name. This organization shall be known as the Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 2. Objects. The objectives of this Section shall be to aid the AMERICAN PHARMACEUTICAL ASSOCIATION in:

1. Encouraging high standards of research in the pharmaceutical sciences.

2. Bringing together, at the Annual Meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION for the mutual benefit and exchange of ideas, those

members and guests of the AMERICAN PHARMACEUTICAL ASSOCIATION who are interested in the pharmaceutical sciences and research as applied to Pharmacy.

3. Representing pharmaceutical sciences and research among the learned societies and aiding in the preparation and distribution of abstracts of papers presented before the Scientific Section.

4. Increasing the value and distribution of the *Scientific Edition* of the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION.

CHAPTER II.—MEMBERSHIP

Article 1. Membership. Attendance upon meetings of the Section and the presentation of competent papers to its programs shall be open to any member of the AMERICAN PHARMACEUTICAL ASSOCIATION. Voting membership shall be limited to regularly enrolled members of the AMERICAN PHARMACEUTICAL ASSOCIATION in good standing,

who have qualified for membership in the Section by supporting, directing, conducting or publishing investigations of a research nature in the pharmaceutical sciences or otherwise providing for education in and dissemination of such investigations.

Applications for membership in the Section shall be made to the Secretary of the Section.

CHAPTER III.—OFFICERS

Article 1. Officers. The officers of the Section shall be a Chairman, a Chairman-Elect, a Vice-Chairman, a Secretary-Treasurer, a Delegate to the House of Delegates of the AMERICAN PHARMACEU-

TICAL ASSOCIATION, and an Alternate Delegate to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION, selected from the voting membership of the Section.

CHAPTER IV.—ELECTION OF OFFICERS

Article 1. Election of Officers. The Chairman shall appoint, any time before or at the first session of the Section, a Nominating Committee of three voting members of the Section. At the final business session of the Section, this Committee shall report one nominee for each office to be filled.

Additional nominations may then be made from the floor. Election shall be conducted by ballot according to parliamentary procedure. The elected members shall hold office until their successors are duly elected and installed.

Article 2. Term of Office. The Secretary-Treasurer and the Delegate to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION shall be elected for terms of three years each. The other officers shall be elected for terms of one year each.

Officers, except the Secretary-Treasurer, may not be re-elected for the same office but may, after a lapse of one term, be eligible again for the same office.

Article 3. Vacancies and Absences from the Annual Meeting. Any vacancies occurring among the officers shall be filled by the Chairman. In the event of the death or resignation of the Chairman, the Chairman-Elect shall assume the duties of that office. In case of the absence of the Chairman from the Annual Meeting of the Section, the Chairman-Elect shall preside. If both the Chairman and the Chairman-Elect are absent, the Vice-Chairman shall preside. In case of absence of the Secretary-Treasurer from the Annual Meeting, he shall send his report and material, if possible, to the Chairman of the Section before the Annual Meeting. The Chairman shall then appoint a Secretary-Treasurer *pro tem* who shall read the report and otherwise carry on the duties of the Secretary-Treasurer during the meetings of the Section. In case of the absence of the Chairman, the Chairman-Elect or the Vice-Chairman or the most recent Past Chairman in attendance shall preside as Chairman in the order here mentioned.

CHAPTER V.—DUTIES OF THE OFFICERS

Article 1. Duties of the Chairman. The chairman shall preside at the business sessions of the Section and shall perform the customary and parliamentary duties established by usage. He shall preside at one of the sessions for the presentation of Research Reports when two or more such sessions are held concurrently.

Article 2. Duties of the Chairman-Elect. The Chairman-Elect shall be Chairman of the Committee on Membership. He shall preside at one of the sessions for the presentation of Research Reports when two or more such sessions are held concurrently. In the absence or temporary incapacity of the Chairman, he shall act as Chairman of the Section.

Article 3. Duties of the Vice-Chairman. The Vice-Chairman shall be a member of the Committee on Membership. He shall preside at one of the sessions for the presentation of Research Reports when more than two such sessions are held concurrently. In the absence or temporary incapacity of the Chairman and the Chairman-Elect, he shall act as Chairman of the Section.

Article 4. Duties of the Secretary-Treasurer. The Secretary-Treasurer shall keep a record of all meetings of the Section, retain copies of the records and documents of the Section and shall transmit these records to the incoming Secretary-Treasurer of the Section. Immediately after the Annual Meeting, the Secretary-Treasurer shall transmit to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, for publication and safe keeping, the minutes of each meeting, together with all documents, reports and papers which belong to the Sec-

tion. He shall transmit to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION the names of the officers elected and the committees appointed, as well as any changes in the personnel of the committees.

He shall send to the members such notices as the business of the Section may require.

Prior to the Annual Meeting, he shall mail to the members of the Section a request for papers and an announcement of the latest date on which they will be accepted for presentation at the Annual Meeting.

He shall plan the program of the Annual Meeting according to Chapter X, Article 7 of these By-Laws. He shall furnish the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION with a copy of the program in time for inclusion in the appropriate number of the JOURNAL preceding the Annual Meeting and in the official program of the meeting.

He shall be responsible for securing and distributing the Scientific Section Abstracts in cooperation with the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Copies of the program and abstracts are to be mailed to the voting members of the Section as early as possible prior to the Annual Meeting.

He shall, as Secretary-Treasurer, prepare and deliver to the Scientific Section an annual report of the activities of his office.

He shall collect the Section dues, transact the fiscal affairs of the Section, pay necessary Section expenses, and keep a record of the finances of the Section.

He shall prepare and transmit the annual report of the Section to the House of Delegates.

CHAPTER VI.—COMMITTEES

Article 1. Standing Committees. The following standing committees, except the Committee on Resolutions, shall be nominated or appointed by the Chairman of the Section after the Annual Meeting in accordance with these By-Laws and the By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION:

1. Committee on Membership
2. Committee on Ebert Prize
3. Committee on Kilmer Prize
4. Committee on Resolutions
5. Auditing Committee

Article 2. Committee on Membership. The Chairman-Elect of the Section shall be Chairman of this Committee. The Vice-Chairman and the Secretary-Treasurer shall be members. There shall be four other members, each representing different phases of pharmaceutical science, of whom two shall be appointed each year from the voting membership for a term of two years.

It shall be the duty of the committee to prepare membership application forms. The committee shall examine all applications for membership submitted

to the Secretary-Treasurer. A majority vote of the committee shall be required to elect an applicant to membership in the Section. The Chairman of the Committee shall prepare an annual report on the status of membership in the Section.

Article 3. Committee on Ebert Prize. The Committee on Ebert Prize shall be nominated by the Chairman of the Section for approval by the Council, as soon as possible after the Annual Meeting in accordance with Chapter IX, Article VII of the By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 4. Committee on Kilmer Prize. The Committee on Kilmer Prize shall be nominated by the Chairman of the Section, for approval by the Council, as soon as possible after the Annual Meeting in accordance with Chapter IX, Article VIII of the By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 5. Committee on Resolutions. The Chairman of the Section shall appoint, at the beginning of the first session, a committee of three members, one of whom shall be designated as

BY-LAWS OF THE SCIENTIFIC SECTION (*Continued*)

Chairman of the Committee. This Committee shall study the recommendations presented in the various reports and other proposals and suggest action to be taken on these by the Section.

Article 6. Auditing Committee. The Chairman of the Section shall appoint a committee of three members, one of whom shall be designated as Chairman of the Committee, for the purpose of auditing the Secretary-Treasurer's accounts.

Article 7. Special Committees. Special committees may be appointed from time to time for special purposes upon recommendation of the Chairman of the Section or upon motion from the floor.

Unless otherwise provided, such Committees shall consist of three members.

Article 8. Executive Committee. The Executive Committee shall consist of the Chairman of the Section, the Chairman-Elect, the Vice-Chairman, the Secretary-Treasurer, and the Delegate to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION, and shall have the authority between regular meetings of the Section to act on such business as the Chairman of the Section and/or the Secretary-Treasurer may place before it. Business of the Committee may be transacted by mail.

CHAPTER VII.—MEETINGS

Article 1. Time of Meeting. The Annual Meeting of the Section shall be held at the same time and place as that of the Annual Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 2. Duration of Sessions and Quorum. The program shall be planned so as to limit each session, if possible, with the exception of the business session, to not more than three hours. Fifteen members shall constitute a quorum.

Article 3. Number of Sessions. The number of sessions to be held at any one Annual Meeting shall be determined by the Secretary-Treasurer in cooperation with the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION. Two or more sessions of the Section for the presentation of papers may be held simultaneously, but only one session of the Section may be held while Section business is being transacted. The sessions shall be so arranged that they will conflict as little as possible with sessions of other Sections, and that no session

shall be held simultaneously with any General Session of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 4. Arrangement of Presentation of Research Reports. In so far as possible, the Secretary-Treasurer in preparing the program shall group Research Reports in related fields.

Article 5. Special Sessions. Special sessions at the Annual Convention may be held at the discretion of the Executive Committee and with the approval of the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION for the purpose of permitting lectures which will require more time than allowed in Chapter X, Article 5.

Article 6. Joint Sessions. Joint sessions of this Section with other Sections may be arranged by the Chairman and the Secretary-Treasurer in cooperation with the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION.

CHAPTER VIII.—ORDER OF BUSINESS

Article 1. The First Session. The order of business at the first business session shall be as follows: Call to Order; Announcement of the Appointment of the Resolutions and Nominating Committees; Chairman's Address; Secretary-Treasurer's Report; Report of the Auditing Committee; Report of Committee on Membership; Report of the Committee on Ebert Prize; Report of the Committee on Kilmer Prize; Reports of Special Committees, and miscellaneous business. The session may then divide into two or more groups for the presentation and discussion of Research Reports.

Article 2. Sessions Other Than the First and Final Business Sessions. Sessions other than the first and final business sessions shall be devoted to the presentation of Research Reports. Provided however, that the discussion of Research Reports

may be interrupted at any time upon call of the Chairman of the Section to convene as a single group to consider matters referred to the Section, by the Council or the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 3. The Final Business Session. The order of business at the final business session shall be as follows: Call to Order; Report of Committee on Resolutions; Call for Unfinished Business; Call for New Business; Report of the Nominating Committee; Election of New Officers; Installation of New Officers, and Adjournment.

Article 4. Suspension of Order. This regular order of business may be suspended at any time during a session of the Section, for that particular session, by a three-fourths vote of the voting membership present.

CHAPTER IX.—EXPENSES

Article 1. Appropriations for Regular Expenses. Appropriations provided by the AMERICAN PHARMACEUTICAL ASSOCIATION to the Section shall be used only to defray expenses for which the appropriation is made.

Article 2. Appropriations for Additional Expenses. Appropriations from the AMERICAN PHARMACEUTICAL ASSOCIATION for expenses other than those provided for in the annual budget of the AMERICAN PHARMACEUTICAL ASSOCIATION, must be procured by authority of the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, on request, through the Secretary-Treasurer of the Section.

Article 3. Dues. Section dues shall be one dollar (\$1.00) per calendar year for all members of the Scientific Section. This shall entitle the member to a copy of the Scientific Section Abstracts; voting privileges; all communications of the Section; the privilege to serve on committees, and to hold any office in the Section. Income from

dues shall be used exclusively for expenses of the Section, as authorized by its Executive Committee and approved by the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, through submission of an annual budget, prepared by the Secretary-Treasurer.

Article 4. Changing of Dues. Proposed changes in the amount of dues for the Section shall be voted upon by the voting members by mail. Any alteration of dues shall require the vote of three-fourths of the members voting and approval by the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 5. Gifts and Donations. Gifts and donations designated for use of the Scientific Section for special purposes may be accepted by the AMERICAN PHARMACEUTICAL ASSOCIATION, with the consent of the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, and with the understanding that they will be administered by the Council.

CHAPTER X.—REPORTS PRESENTED BEFORE THE SCIENTIFIC SECTION

Article 1. Definition of Abstract of Research. For the purpose of this Section, the term "abstract" shall mean either of the following: Type I—an abstract of a description of completed research; Type II—a progress report on an incomplete project. For the purpose of printing in the Scientific Section abstracts, such abstracts should be 150-200 words in length.

Article 2. Presentation of Research. For the purpose of the Section, the term "presentation" of research shall mean the oral report given at one of the sessions of the Section. Two kinds (Type I and Type II) of Research Reports may be accepted for presentation before the Section.

Members of the Section may submit Research Reports and present them at the sessions, and other research workers, whether members of the AMERICAN PHARMACEUTICAL ASSOCIATION or not, may also present Research Reports to the Section, but these reports will be marked in such a manner in the program to indicate that the author or authors are nonmembers.

Article 3. Rules Governing the Submission of Type I Research Reports. An abstract (Article 1) shall be sent to the Secretary-Treasurer of the Section. An original and one copy of the completed paper shall be turned over to the Secretary-Treasurer when the oral report is presented.

Article 4. Rules Governing the Submission of Type II Research Reports. An abstract (Article 1) shall be sent to the Secretary-Treasurer of the Section. No copy of a written paper shall be required.

Article 5. Rules Governing the Presentation of Research Reports. The time allowed for the presentation of a report shall be determined by the Chairman of the Section after consultation with the Secretary-Treasurer. The time allowed each

speaker in the discussion of a report and the total time allowed for the discussion of any report shall be determined by the Chairman of the Section.

Article 6. Special Papers and Guest Speakers. From time to time outstanding scientists working in fields of interest to the Section may be invited to prepare and present papers to the Section. Any member may suggest such a guest speaker, but the suggestion must be approved by the Executive Committee of the Section and an official invitation extended by the Chairman.

Article 7. Program. The Secretary-Treasurer shall prepare a "Program of the Annual Meeting of the Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION," which shall include the date and time of meeting of all the sessions of the Section; order of business; and titles and authors of papers. When a Research Report has more than one author, the name of the author who is to present the report shall be indicated in some manner (*italics* or underlined). When a Research Report is given by a non-member, it shall be indicated as specified in Chapter X, Article 2. The program shall be planned according to Chapter VII, Articles 1, 2, 3 and 4; and Chapter VIII, Articles 1, 2 and 3.

Article 8. Ownership of Papers and Reports. Abstracts, as defined in Chapter X, Article 1, shall become the property of the AMERICAN PHARMACEUTICAL ASSOCIATION for publication in the Scientific Section Abstracts. The completed paper of Type I Research Reports shall become the property of the AMERICAN PHARMACEUTICAL ASSOCIATION, but upon request to the AMERICAN PHARMACEUTICAL ASSOCIATION may be released to the author for publication in other journals, in accordance with Chapter V, Article 7, of the By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION.

BY-LAWS OF THE SCIENTIFIC SECTION (*Continued*)

CHAPTER XI.—AMENDMENTS

Article 1. Amendments. These By-Laws may be amended at any business session of the Section at the Annual Meeting by a two thirds vote of the voting members present, provided notice of such amendment is given together with the text thereof at any previous session held at that Annual Meeting

Amendments may also be approved by mail ballot by two thirds of those voting. Amendments must finally be accepted by the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION as not in conflict with the Constitution and By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION.

CHAPTER XII

Article 1. Miscellaneous. Questions not specifically covered by these By-Laws must always be

decided in accord with the Constitution and By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION

AMERICAN PHARMACEUTICAL ASSOCIATION

OFFICERS 1957-58

President, Joseph B Burt, Lincoln, Neb
Honorary President, Frank O Taylor, South Fort Mitchell, Ky
First Vice President, J Warren Lansdowne, Indianapolis, Ind
Second Vice President, Leroy A Weidle, Sr, St Louis, Mo
Secretary, Robert P Fischelis, Washington, D C
Treasurer, Hugo H Schaefer, Yonkers, N Y

Ex-Officio Members—Joseph B Burt, Lincoln, Neb, Robert P Fischelis, Washington, D C, Nicholas S Gesualde, New York, N Y, J Warren Lansdowne, Indianapolis, Ind, John A MacCartney, Detroit, Mich, Hugo H Schaefer, Yonkers, N Y, Leroy A Weidle, Sr, St Louis, Mo

OFFICERS-ELECT 1958-59

(*Term of office begins April 26, 1958*)

President Elect, Louis J Fischl, Oakland, Calif
First Vice President Elect, Stephen Wilson, Detroit, Mich
Second Vice President Elect, Howell R Jordan, Austin, Tex
Council Members Elect (1961) Troy C Daniels, San Francisco, Calif, J H F Dunning, Baltimore, Md, John A MacCartney, Detroit, Mich

OFFICERS OF THE COUNCIL

Chairman, John B Heinz, Salt Lake City, Utah
Vice Chairman, Henry H Gregg, Minneapolis, Minn
Secretary, Robert P Fischelis, Washington, D C

OFFICERS OF THE HOUSE OF DELEGATES

Chairman, Nicholas S Gesualde, Great Neck, Long Island, N Y
Vice Chairman, Ewart A Swinyard, Salt Lake City, Utah

THE COUNCIL

Elected Members—George F Archambault, Bethesda, Md (1959), Roy A Bowers, Newark, N J (1960), Grover C Bowles, Memphis, Tenn (1960), H A B Dunning, Baltimore, Md (1958), Louis J Fischl, Oakland, Calif (1958), F Royce Franzoni, Arlington, Va (1959), Henry H Gregg, Minneapolis, Minn (1958), John B Heinz, Salt Lake City, Utah (1960), Robert L Swain, New York, N Y (1959)

Editors of the Journal

Scientific Edition, Justin L Powers, Washington, D C
Practical Pharmacy Edition, Eric W Martin, Washington, D C

BY-LAWS OF THE SECTION ON PRACTICAL PHARMACY

CHAPTER I.—NAME AND OBJECTS

Article 1. Name. This organization shall be known as the *Section on Practical Pharmacy* of the AMERICAN PHARMACEUTICAL ASSOCIATION

Article 2. Objects The objectives of this Section shall be to aid the AMERICAN PHARMACEUTICAL ASSOCIATION in

1 Encouraging high standards of research in Pharmacy

2 Bringing together, at the Annual Meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION for the mutual benefit and exchange of ideas, *practicing*

pharmacists, members of educational and industrial organizations, and all others interested in the advancement of pharmacy

3 Representing pharmaceutical sciences and research among the learned societies and aiding in the preparation and distribution of abstracts of papers presented before the *Section on Practical Pharmacy*

4 Increasing the value and distribution of the *Practical Pharmacy Edition* of the Journal of the AMERICAN PHARMACEUTICAL ASSOCIATION

CHAPTER II.—MEMBERSHIP

Article 1. Membership. Attendance at meetings of the Section and the presentation of competent papers to its programs shall be open to any member of the AMERICAN PHARMACEUTICAL ASSOCIATION Voting membership shall be limited to regularly en-

rolled members of the AMERICAN PHARMACEUTICAL ASSOCIATION in good standing, *who are also members of the Section*

Applications for membership in the Section shall be made to the Secretary of the Section

CHAPTER III.—OFFICERS

Article 1. Officers. The officers of the Section shall be a Chairman, a Chairman Elect, a Vice-Chairman, a Secretary Treasurer, a Delegate to the House of Delegates of the AMERICAN PHARMACEUTI

CAL ASSOCIATION, and an Alternate Delegate to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION, selected from the voting membership of the Section

CHAPTER IV.—ELECTION OF OFFICERS

Article 1. Election of Officers. The Chairman shall appoint, any time before or at the first session of the Section a Nominating Committee of three voting members of the Section At the final business session of the Section, this Committee shall report one nominee for each office to be filled Additional nominations may then be made from the floor Election shall be conducted by ballot according to parliamentary procedure The elected members shall hold office until their successors are duly elected and installed

Article 2. Term of Office. The Secretary-Treasurer and the Delegate to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION shall be elected for terms of three years each The other officers shall be elected for terms of one year each

Officers, except the Secretary-Treasurer, may not be reelected for the same office after completing one term of service but may, after a lapse of two terms, be eligible again for office

Article 3. Vacancies and Absences from the Annual Meeting. Any vacancies occurring among the officers shall be filled by appointment by the Chairman In the event of the death or resignation of the Chairman, the Chairman-Elect shall assume the duties of that office In case of the absence of the Chairman from the Annual Meeting of the Section, the Chairman-Elect shall preside If both the Chairman and the Chairman-Elect are absent, the Vice Chairman shall preside In case of absence of the Secretary-Treasurer from the Annual Meeting, he shall send his report and material, if possible, to the Chairman of the Section before the Annual Meeting The Chairman shall then appoint a Secretary-Treasurer *pro tem* who shall read the report and otherwise carry on the duties of the Secretary-Treasurer during the meetings of the Section In case of the absence of the Chairman, the Chairman Elect and the Vice-Chairman, the most recent Past Chairman in attendance shall preside

CHAPTER V.—DUTIES OF THE OFFICERS

Article 1. Duties of the Chairman. The Chairman shall preside at the business sessions of the Section and shall perform the customary and parliamentary duties established by usage He shall preside at one of the sessions for the presentation of Re-

search Reports when two or more such sessions are held concurrently

Article 2. Duties of the Chairman-Elect. The Chairman-Elect shall be chairman of the Committee on Membership He shall preside at one of the

sessions for the presentation of Research Reports when two or more such sessions are held concurrently. In the absence or temporary incapacity of the Chairman, he shall act as Chairman of the Section.

Article 3. Duties of the Vice-Chairman. The Vice-Chairman shall be a member of the Committee on Membership. He shall preside at one of the sessions for the presentation of Research Reports when more than two such sessions are held concurrently. In the absence or temporary incapacity of the Chairman and the Chairman-Elect, he shall act as Chairman of the Section.

Article 4. Duties of the Secretary-Treasurer. The Secretary-Treasurer shall keep a record of all meetings of the Section, retain copies of the records and documents of the Section and shall transmit these records to the incoming Secretary-Treasurer of the Section. Immediately after the Annual Meeting, the Secretary-Treasurer shall transmit to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, for publication and safekeeping, the minutes of each meeting, together with all documents, reports and papers which belong to the Section. He shall transmit to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION the names of the officers elected and the committees appointed, as well as any

changes in the personnel of the committees.

He shall send to the members such notices as the business of the Section may require.

Prior to the Annual Meeting, he shall mail to the members of the Section a request for papers and an announcement of the latest date on which they will be accepted for presentation at the Annual Meeting.

He shall plan the program of the Annual Meeting according to Chapter X, Article 7 of these By-Laws. He shall furnish the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION with a copy of the program in time for its inclusion in the appropriate number of the Journal preceding the Annual Meeting.

Copies of the program and abstracts are to be mailed to the voting members of the Section as early as possible prior to the Annual Meeting.

He shall, as Secretary-Treasurer, prepare and deliver to the Section an annual report of the activities of his office.

He shall collect the Section dues, transact the fiscal affairs of the Section, pay necessary Section expenses, and keep a record of the finances of the Section.

He shall prepare and transmit *through the official delegate of the Section* the annual report of the Section to the House of Delegates.

CHAPTER VI.—COMMITTEES

Article 1. Committees. The following standing Committees, except the Committee on Auditing, shall be appointed by the Chairman of the Section after the Annual Meeting in accordance with these By-Laws and the By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION:

1. Membership
2. Resolutions
3. Auditing

Article 2. Committee on Membership.

The Chairman-Elect of the Section shall be Chairman of this Committee. The Vice-Chairman and the Secretary-Treasurer shall be members. There shall be four other members, appointed from the voting membership, two of whom shall be appointed each year for a term of two years.

It shall be the duty of the committee to prepare membership application forms. The committee shall review all applications for membership submitted to the Secretary-Treasurer and the Chairman of the committee shall prepare an annual report on the status of membership in the Section.

Article 3. Committee on Resolutions. The Chairman of the Section shall appoint, at the beginning of the first session, a committee of three members, one of whom shall be designated as Chairman of the Committee. This Committee shall study the recommendations presented in the various reports and

other proposals and suggest action to be taken on these by the Section.

Article 4. Auditing Committee. The Chairman of the Section shall appoint a committee of three members, one of whom shall be designated as Chairman of the Committee, for the purpose of auditing the Secretary-Treasurer's accounts. *This Committee shall be appointed at least four months prior to the Annual Meeting.*

Article 5. Special Committees. Special committees such as *Nominating Committee, Committee on Constitution and By-Laws*, and others may be appointed from time to time for special purposes upon recommendation of the Chairman of the Section or upon motion from the floor. Unless otherwise provided, such Committees shall consist of three members.

Article 6. Executive Committee. The Executive Committee shall consist of the Chairman of the Section, the Chairman-Elect, the Vice-Chairman, the Secretary-Treasurer, and the Delegate to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION, and shall have the authority between regular meetings of the Section to act on such business as the Chairman of the Section and/or the Secretary-Treasurer may place before it. Business of the Committee may be transacted by mail.

CHAPTER VII.—MEETINGS

Article 1. Time of Meeting. The Annual Meeting of the Section shall be held at the same time and place as that of the Annual Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 2. Duration of Sessions and Quorum. The program shall be planned so as to limit each session, if possible, with the exception of the business session, to not more than three hours. Fifteen members shall constitute the quorum.

Article 3. Number of Sessions. The number of sessions to be held at any one Annual Meeting shall be determined by the Secretary-Treasurer in cooperation with the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 4. Arrangement of Presentation of Re-

search Reports. Insofar as possible, the Secretary-Treasurer in preparing the program shall group Research Reports in related fields.

Article 5. Special Sessions. Special sessions at the annual convention may be held at the discretion of the Executive Committee and with the approval of the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION for the purpose of permitting lectures which will require more time than allowed in Chapter X, Article 5.

Article 6. Joint Sessions. Joint sessions of this Section with other Sections may be arranged by the Chairman and the Secretary-Treasurer in cooperation with the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION.

CHAPTER VIII.—ORDER OF BUSINESS

Article 1. The First Session. The order of business at the first session shall be as follows: Call to Order; announcement of the appointment of the Resolutions and Nominating Committees; Chairman's Address; Secretary-Treasurer's Report; Report of the Auditing Committee; Report of the Committee on Membership; Reports of Special Committees, and miscellaneous business. The Section may then divide into two or more groups for the presentation and discussion of Research Reports.

Article 2. Sessions Other Than the First and Final Business Sessions. Sessions other than the first and final business sessions shall be devoted to

the presentation of Research Reports.

Article 3. The Final Business Session. The order of business at the final business session shall be as follows: Call to Order; Report of the Committee on Resolutions; Call for Unfinished Business; Call for New Business; Report of the Nominating Committee; Election of New Officers; Installation of Officers, and Adjournment.

Article 4. Suspension of Order. This regular order of business may be suspended at any time during a session of the Section, for that particular session, by a three-fourths vote of the voting membership present.

CHAPTER IX.—EXPENSES

Article 1. Appropriations for Regular Expenses. Appropriations provided by the AMERICAN PHARMACEUTICAL ASSOCIATION to the Section shall be used only to defray expenses for which the appropriation is made.

Article 2. Appropriations for Additional Expenses. Appropriations from the AMERICAN PHARMACEUTICAL ASSOCIATION for expenses other than those provided for in the annual budget of the AMERICAN PHARMACEUTICAL ASSOCIATION, must be procured by authority of the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, on request, through the Secretary-Treasurer of the Section.

Article 3. Dues. Section dues shall be one dollar (\$1.00) per calendar year for all members of the *Section on Practical Pharmacy*. This shall entitle

the member to a copy of the Section Abstracts; voting privileges; all communications of the Section; the privilege to serve on committees, and to hold any office in the Section. Income from dues shall be used exclusively for expenses of the Section, as authorized by its Executive Committee and approved by the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, through submission of an annual budget, prepared by the Secretary-Treasurer.

Article 4. Gifts and Donations. Gifts and donations designated for use of the Section for special purposes may be accepted by the AMERICAN PHARMACEUTICAL ASSOCIATION, with the consent of the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION, and with the understanding that they will be administered by the Council.

CHAPTER X.—REPORTS PRESENTED BEFORE THE SECTION ON PRACTICAL PHARMACY

Article 1. Definition of Abstract of Research. For the purpose of this Section, the term "abstract" shall mean either of the following: Type I—An abstract of a description of completed research; Type II—A progress report on an incomplete project. For the purpose of printing in the Section Abstracts, such abstracts should be 150–200 words in length.

Article 2. Presentation of Research. For the purpose of the Section, the term "presentation" of research shall mean the oral report given at one of the sessions of the Section. Two kinds—(Type I and Type II) of Research Reports may be accepted for presentation before the Section.

Members of the Section may submit Research

Reports and present them at the sessions, and other research workers, whether members of the AMERICAN PHARMACEUTICAL ASSOCIATION or not, may also present Research Reports to the Section, but these reports will be marked in such a manner in the program to indicate that the author or authors are non-members of the AMERICAN PHARMACEUTICAL ASSOCIATION.

Article 3. Rules Governing the Submission of Type I Research Reports. An abstract (Article 1) shall be sent *by the author or authors* to the Secretary-Treasurer of the Section *prior to the deadline date which shall be specified in the Secretary's request for papers*. An original and one copy of the completed paper shall be submitted to the Secretary, *prior to or at the time the oral report is presented*.

Article 4. Rules Governing the Submission of Type II Research Reports. An abstract (Article 1) shall be sent to the Secretary-Treasurer of the Section *prior to the deadline date specified by the Secretary-Treasurer of the Section in his request for papers*. No copy of the written paper shall be required *unless the author or authors wish to have their work published as a note in the Journal*.

Article 5. Rules Governing the Presentation of Research Reports. The time allowed for the presentation of a report shall be determined by the Chairman of the Section after consultation with the Secretary-Treasurer. The time allowed each speaker in the discussion of a report and the total time allowed each speaker in the discussion of a report and the total time allowed for the discussion of any report shall be determined by the Chairman of the Section.

Article 6. Special Papers and Guest Speakers. Any member may suggest such a guest speaker, but the suggestion must be approved by the Executive Committee of the Section and an official invitation will then be extended by the Chairman or the Secretary of the Section.

Article 7. Program. The Secretary-Treasurer shall prepare a "Program of the Annual Meeting of the Section on Practical Pharmacy of the AMERICAN PHARMACEUTICAL ASSOCIATION," which shall include the date and time of meeting of all the sessions of the Section; order of business; and titles and authors of papers. When a Research Report has more than one author, the name of the author who is to present the report shall be indicated in some manner (italics or underlined). When a Research Report is given by a non-member, it shall be indicated as specified in Chapter X, Article 2. The program shall be planned according to Chapter VII, and Chapter VIII, Articles 1, 2, and 3.

Article 8. Ownership of Papers and Reports. Abstracts, as defined in Chapter X, Article 1, shall become the property of the AMERICAN PHARMACEUTICAL ASSOCIATION, for publication in the Section on Practical Pharmacy Abstracts. The completed paper on Type I Research Reports shall become the property of the AMERICAN PHARMACEUTICAL ASSOCIATION, but upon request to the AMERICAN PHARMACEUTICAL ASSOCIATION it may be released to the author for publication in other journals, in accordance with Chapter V, Article 7, of the By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION.

CHAPTER XI.—AMENDMENTS

Article 1. Amendments. These By-Laws may be amended at any business session of the Section at the Annual Meeting by a two-thirds vote of the voting members present, provided notice of such amendment is given together with the text thereof at any previous session held at that Annual Meeting.

Amendments may also be approved by mail ballot by two-thirds of those voting. Amendments must finally be accepted by the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION as not in conflict with the Constitution and By-Laws of the AMERICAN PHARMACEUTICAL ASSOCIATION.

BY-LAWS OF THE STUDENT SECTION OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

(With all amendments to December 31, 1957)

CHAPTER I—NAME AND OBJECTS

Article 1. Name. This organization shall be known as the Student Section of the American Pharmaceutical Association.

Article 2. Objects. The objectives of this Section shall be to aid the American Pharmaceutical Association in:

1. Promoting the interest of associate members in the American Pharmaceutical Association.

2. Bringing together at the Annual Meeting of the American Pharmaceutical Association those students and representatives of Student Branches in attendance for the purpose of discussing matters of

common interest and encouraging their participation in the program of the Association.

3. Providing an avenue whereby appropriate resolutions pertaining to student affairs may be submitted to the House of Delegates of the American Pharmaceutical Association for its consideration.

4. Strengthening the program whereby associate members, upon their graduation from college, become full members of the Association and continue to participate in its program for the advancement of pharmacy.

CHAPTER II—MEMBERSHIP

Article 1. Membership and Voting. Attendance upon meetings of the Section shall be open to any member or associate member of the American Pharmaceutical Association. All associate members

in good standing shall automatically be considered members of this Section.

All voting shall be by branches and each branch represented at the meetings shall be entitled to one vote.

CHAPTER III—OFFICERS

Article 1. Officers. The officers of the Section shall be a Chairman, a Vice-Chairman, a Secretary-Treasurer, a Delegate to the House of Delegates of the American Pharmaceutical Association, and three Alternate Delegates to the House of Delegates of the American Pharmaceutical Association, each selected from the members of the Section.

Article 2. Voting Delegate. A voting delegate shall be a member in good standing of a Student Branch of the American Pharmaceutical Association who has been appointed or elected by his or her Branch, in good standing, to represent his or her Branch in all matters pertaining to the Student Section.

CHAPTER IV—ELECTION OF OFFICERS

Article 1. Election of Officers. The Chairman shall appoint, any time before or at the first session of the Section, a nominating committee of three members of the Section. At the final business session of the Section, this committee shall report one nominee for each office to be filled.

Additional nominations may then be made from the floor. Election shall be conducted by ballot according to parliamentary procedure. The elected members shall hold office until their successors are duly elected and installed.

Article 2. Vacancies and Absences from the Annual Meeting. Any vacancies occurring among the officers shall be filled by the Chairman. In the event of the death, resignation, or absence from the

Annual Meeting of the Chairman, the Vice-Chairman shall assume the duties of that office. In the event that neither the Chairman nor Vice-Chairman can attend the Annual Meeting, a Chairman *pro tem* shall be appointed to preside by the Chairman of the Committee on Student Branches of the American Pharmaceutical Association. In case of absence of the Secretary-Treasurer from the Annual Meeting, he shall send his report and material to the Chairman of the Section before the Annual Meeting. The Chairman shall then appoint a Secretary-Treasurer *pro tem* who shall read the report and otherwise carry on the duties of the Secretary-Treasurer during the meetings of the Section.

CHAPTER V—DUTIES OF THE OFFICERS

Article 1. Duties of the Chairman. The Chairman shall preside at the business sessions of the Section and shall perform the customary and parliamentary duties established by usage. He shall also

assist the Secretary-Treasurer in his duties of arranging the program for the Section at the Annual Meeting.

BY-LAWS OF THE STUDENT SECTION (*Continued*)

Article 2. Duties of the Vice-Chairman. The Vice-Chairman shall preside at those sessions of the Section when the Chairman is either absent or temporarily incapacitated.

Article 3. Duties of the Secretary-Treasurer. The Secretary-Treasurer shall keep a record of all meetings of the Section, retain copies of the records and documents of the Section and shall transmit these records to the incoming Secretary-Treasurer of the Section. Immediately after the Annual Meeting, the Secretary-Treasurer shall transmit to the Secretary of the American Pharmaceutical Association, for publication and safe keeping, the minutes of each meeting, together with all documents, reports and papers which belong to the Section. He shall transmit to the Secretary of the American Pharmaceutical Association the names of the officers

elected and the committees appointed, as well as any changes in the personnel of the committees.

He shall send to the members such notices as the business of the Section may require.

Prior to the Annual Meeting, he shall mail to the members of the Section a request for papers and an announcement of the latest date on which they will be accepted for presentation at the Annual Meeting.

He shall, as Secretary-Treasurer, prepare and deliver to the Section an annual report of the activities of his office.

He shall collect the Section dues, transact the fiscal affairs of the Section, pay necessary Section expenses, and keep a record of the finances of the Section.

He shall prepare and transmit the annual report of the Section to the House of Delegates.

CHAPTER VI—COMMITTEES

Article 1. Committees. The following committees shall be appointed by the Chairman of the Section at the first session of the Section at the Annual Meeting:

1. Committee on Nominations
2. Committee on Resolutions
3. Auditing Committee
4. Committee on Constitution and By-Laws

Article 2. Committee on Nominations. The Committee on Nominations shall present a ballot giving one nominee for each office of the Section at the final business session at the Annual Meeting.

Article 3. Committee on Resolutions. The Committee on Resolutions shall study the recom-

mendations presented in reports and/or by members of the Section and suggest action to be taken on these by the Section.

Article 4. Auditing Committee. The Auditing Committee shall audit the accounts of the Secretary-Treasurer and make its report at the last session of the Section.

Article 5. Committee on Constitution and By-Laws. The Committee on Constitution and By-Laws shall study and formalize the notices of any amendments presented in accordance with Chapter IX, Article 1, and prepare the amendments for presentation to the Section.

CHAPTER VII—MEETINGS

Article 1. Time of Meeting. The Annual Meeting of the Section shall be held at the same time and place as that of the Annual Convention of the American Pharmaceutical Association.

Article 2. Duration of Sessions and Quorum. The program shall be planned so as to limit each session, if possible, to not more than three hours. Fifteen members shall constitute a quorum.

Article 3. Number of Sessions. The number of sessions to be held at any one Annual Meeting shall be determined by the Secretary-Treasurer in cooperation with the Secretary of the American Pharmaceutical Association. The sessions shall be so arranged that they will conflict as little as possible with sessions of other Sections, and that no session shall be held simultaneously with any General Session of the American Pharmaceutical Association.

CHAPTER VIII—EXPENSES

Article 1. Appropriations. Appropriations provided by the American Pharmaceutical Association to the Section shall be used only to defray expenses for which the appropriation is made.

Article 2. Dues. There shall be no dues required of individual members of the Section but each Student Branch of the American Pharmaceutical Association will be asked to make a contribution of \$10.00 (ten dollars) *per annum* in support of the Section.

Article 3. Expenses of Officers. Where considered expedient and necessary, the expenses of the Chairman and the Secretary-Treasurer, incurred in attending the Annual Meeting, would be paid from the funds of the Section. This expenditure shall be subject to the approval of the Secretary and General Manager of the American Pharmaceutical Association.

CHAPTER IX—AMENDMENTS

Article 1. Amendments. These By-Laws may be amended at any business session of the Section at the Annual Meeting by a two-thirds vote of the members present, provided notice of such amendment is given together with the text thereof at any previous session held at that Annual Meeting.

Amendments may also be approved by mail ballot by two-thirds of those voting. Amendments must finally be accepted by the Council of the American Pharmaceutical Association as not in conflict with the Constitution and By-Laws of the American Pharmaceutical Association.

CHAPTER X

Article 1. Miscellaneous. Questions not specifically covered by these By-Laws must always be

decided in accord with the Constitution and By-Laws of the American Pharmaceutical Association.

AMERICAN PHARMACEUTICAL ASSOCIATION

CERTIFICATE OF INCORPORATION

WHEREAS, we, the undersigned, desire to form an association having for its object to unite the educated and reputable Pharmacists and Druggists of America, as will more fully hereinafter appear;

Now, therefore, we do hereby certify as follows:

First: The corporate name of the ASSOCIATION is the AMERICAN PHARMACEUTICAL ASSOCIATION.

Second: The ASSOCIATION shall continue until dissolved by the action of its members, or by the operation of law.

Third: The objects and business of said ASSOCIATION are as follows:

(a) To improve and regulate the drug market, by preventing the importation of inferior, adulterated or deteriorated drugs, and by detecting and exposing home adulterations.

(b) To encourage proper relations between Druggists, Pharmacists, Physicians, and the people at large, which shall promote the public welfare, and tend to mutual strength and advantage.

(c) To improve the science and art of Pharmacy by diffusing scientific knowledge among Apothecaries and Druggists, fostering pharmaceutical literature, developing talent, stimulating discovery and invention, and in encouraging home production and manufacture in the several departments of the drug business.

(d) To regulate the system of apprenticeship and employment, so as to prevent, so far as possible, the evils flowing from deficient training in the responsible duties of preparing, dispensing and selling medicines.

(e) To suppress empiricism, and to restrict the dispensing and sale of medicines to regularly educated Druggists and Apothecaries.

(f) To uphold standards of authority in the education, theory and practice of Pharmacy.

(g) To create and maintain a standard of professional honesty equal to the amount of our professional knowledge, with a view to the highest good and the greatest protection to the public.

Fourth: The concerns and affairs of the ASSOCIATION shall be managed by a Council, which shall

consist for the first year of John U. Lloyd, Maurice W. Alexander, Alexander K. Finlay, Karl Simmon, Samuel A. D. Sheppard, John M. Maisch, James Vernor, C. Lewis Diehl, William H. Rogers, William Saunders, Albert E. Ebert, Philip C. Candidus, George W. Kennedy, Albert H. Hollister, James M. Good, Lewis C. Hopp and William Dupont.

Given under our respective hands and seals this 12th day of December, A.D. 1887.

Signed:

JOHN U. LLOYD,
ALEX. K. FINLAY,
SAMUEL A. D. SHEPPARD,
JAMES VERNOR,
WILLIAM H. ROGERS,
ALBERT E. EBERT,
GEORGE W. KENNEDY,
PHILIP C. CANDIDUS,
ALBERT H. HOLLISTER,
LEWIS C. HOPP,
JOHN A. MILBURN,
E. B. BURY,
W. S. THOMPSON,
CHARLES CHRISTIANI,
A. J. SCHAFHIRT,
O. H. COUMBE,
GEO. B. LOCKHART,
T. C. MURRAY,
JOSEPH R. WALTON,
G. G. C. SIMMS,

JAMES M. GOOD,
MAURICE W. ALEXAN-
DER,
KARL SIMMON,
JOHN M. MAISCH,
C. LEWIS DIEHL,
WM. SAUNDERS,
WILLIAM DUPONT,
Members of the Council.
and
Z. W. CROMWELL,
JOHN R. MAJOR,
W. G. DUCKETT,
GEO. W. BOYD,
HENRY A. JOHNSTON,
W. C. MILBURN,
ARTHUR NATTANS,
THOMAS M. WEHRLY,
of the District of
Columbia

(Notaries' certificates attached to the original document attest the genuineness of each and every signature.)

Received for record February 21, 1888, at 1:05 P.M., and recorded in Liber No. 4, fol. 302 Acts of Incorporation, District of Columbia, and examined.

JAMES M. TROTTER, *Recorder.*

Signed:

SEAL:

Office of Recorder of Deeds,
District of Columbia,
Washington, D. C.

LIST OF OFFICERS OF THE ASSOCIATION SINCE ITS ORGANIZATION

Names of deceased officers in italics.

PRESIDENTS AND LOCAL SECRETARIES

Year	Place of Meeting and Date	Presidents and Local Secretaries
1852	Philadelphia, Pa., Oct. 6, 1852	Organization Meeting.
1852-53	Boston, Mass., Aug. 24, 1853	<i>Daniel B. Smith</i> , Philadelphia, Pa.
1853-54	Cincinnati, O., July 25, 1854	<i>William A. Brewer</i> , Boston, Mass.
1854-55	New York, N. Y., Sept. 11, 1855	<i>William B. Chapman</i> , Cincinnati, O.
1855-56	Baltimore, Md., Sept. 9, 1856	<i>John Meakin</i> , New York, N. Y.
1856-57	Philadelphia, Pa., Sept. 8, 1857	<i>George W. Andrews</i> , Baltimore, Md.
1857-58	Washington, D. C., Sept. 14, 1858	<i>Charles Ellis</i> , Philadelphia, Pa.
1858-59	Boston, Mass., Sept. 13, 1859	<i>John I. Kidwell</i> , Georgetown, D. C.
1859-60	New York, N. Y., Sept. 11, 1860	<i>Samuel M. Colcord</i> , Boston, Mass.
1860-62	Philadelphia, Pa., Aug. 27, 1862	<i>Henry T. Kiersted</i> , New York, N. Y.
1862-63	Baltimore, Md., Sept. 8, 1863	<i>Wm. Procter, Jr.</i> , Philadelphia, Pa.
1863-64	Cincinnati, O., Sept. 21, 1864	<i>J. Faris Moore</i> , Baltimore, Md.
1864-65	Boston, Mass., Sept. 5, 1865	<i>William J. M. Gordon</i> , Cincinnati, O.
1865-66	Detroit, Mich., Aug. 22, 1866	<i>Henry W. Lincoln</i> , Boston, Mass.
1866-67	New York, N. Y., Sept. 10, 1867	<i>Frederick Stearns</i> , Detroit, Mich., <i>P. Wendover Bedford</i>
1867-68	Philadelphia, Pa., Sept. 8, 1868	<i>John Milhau</i> , New York, N. Y., <i>Alfred B. Taylor</i>
1868-69	Chicago, Ill., Sept. 7, 1869	<i>Edward Parrish</i> , Philadelphia, Pa., <i>Henry W. Fuller</i>
1869-70	Baltimore, Md., Sept. 13, 1870	<i>Ezekiel H. Sargent</i> , Chicago, Ill., <i>J. Faris Moore</i>
1870-71	St. Louis, Mo., Sept. 12, 1871	<i>Richard H. Stabler</i> , Alexandria, Va., <i>William H. Crawford</i>
1871-72	Cleveland, O., Sept. 3, 1872	<i>Enno Sander</i> , St. Louis, Mo., <i>Henry C. Gaylord</i>
1872-73	Richmond, Va., Sept. 16, 1873	<i>Albert E. Ebert</i> , Chicago, Ill., <i>Thomas H. Hazard</i>
1873-74	Louisville, Ky., Sept. 8, 1874	<i>John F. Hancock</i> , Baltimore, Md., <i>Emil Scheffer</i>
1874-75	Boston, Mass., Sept. 7, 1875	<i>C. Lewis Diehl</i> , Louisville, Ky., <i>Samuel A. D. Sheppard</i>
1875-76	Philadelphia, Pa., Sept. 12, 1876	<i>George F. H. Markoe</i> , Boston, Mass., <i>Adolphus W. Miller</i>
1876-77	Toronto, Can., Sept. 4, 1877	<i>Charles Bullick</i> , Philadelphia, Pa., <i>Henry J. Rose</i>
1877-78	Atlanta, Ga., Nov. 26, 1878	<i>William Saunders</i> , London, Ont., <i>Jesse W. Rankin</i>
1878-79	Indianapolis, Ind., Sept. 9, 1879	<i>Gustavus J. Luhn</i> , Charleston, S. C., <i>Eli Lilly</i>
1879-80	Saratoga, N. Y., Sept. 14, 1880	<i>George W. Sloan</i> , Indianapolis, Ind., <i>Charles F. Fish</i>
1880-81	Kansas City, Mo., Aug. 23, 1881	<i>James T. Shinn</i> , Philadelphia, Pa., <i>William T. Ford</i>
1881-82	Niagara Falls, N. Y., Sept. 12, 1882	<i>P. Wendover Bedford</i> , New York, N. Y., <i>Hiram E. Griffith</i>
1882-83	Washington, D. C., Sept. 11, 1883	<i>Charles A. Heinisch</i> , Lancaster, Pa., <i>Charles Becker</i>
1883-84	Milwaukee, Wis., Aug. 26, 1884	<i>Wm. S. Thompson</i> , Washington, D. C., <i>Henry C. Schranck</i>
1884-85	Pittsburgh, Pa., Sept. 8, 1885	<i>John Ingalls</i> , Macon, Ga., <i>George A. Kelley</i>
1885-86	Providence, R. I., Sept. 7, 1886	<i>Joseph Roberts</i> , Baltimore, Md., <i>William B. Blanding</i>
1886-87	Cincinnati, O., Sept. 5, 1887	<i>Chas. A. Tufts</i> , Dover, N. H., <i>George W. Voss</i>
1887-88	Detroit, Mich., Sept. 3, 1888	<i>John U. Lloyd</i> , Cincinnati, O., <i>James Vernor</i>
1888-89	San Francisco, Cal., June 24, 1889	<i>M. W. Alexander</i> , St. Louis, Mo., <i>Edward W. Runynn</i>
1889-90	Old Point Comfort, Va., Sept. 8, 1890	<i>Emlen Painter</i> , New York, N. Y., <i>Charles E. Dohme</i>
1890-91	New Orleans, La., April 27, 1891	<i>A. B. Taylor</i> , Philadelphia, Pa., <i>A. K. Finlay</i>
1891-92	Profile House, N. H., July 14, 1892	<i>A. K. Finlay</i> , New Orleans, La., <i>H. M. Whitney</i>
1892-93	Chicago, Ill., Aug. 14, 1893	<i>Jos. P. Remington</i> , Philadelphia, Pa., <i>Henry Biroth</i>
1893-94	Asheville, N. C., Sept. 3, 1894	<i>Edgar L. Patch</i> , Boston, Mass., <i>W. G. Smith</i>
1894-95	Denver, Colo., Aug. 14, 1895	<i>William Simpson</i> , Raleigh, N. C., <i>Edm. L. Scholtz</i>
1895-96	Montreal, Can., Aug. 12, 1896	<i>James M. Good</i> , St. Louis, Mo., <i>Joseph E. Morrison</i>
1896-97	Lake Minnetonka, Minn., Aug. 23, 1897	<i>Joseph E. Morrison</i> , Montreal, Can., <i>Edw. Shumpik</i>
1897-98	Baltimore, Md., Aug. 20, 1898	<i>Henry M. Whitney</i> , Lawrence, Mass., <i>Henry P. Hynson</i>
1898-99	Put-in-Bay, O., Sept. 4, 1899	<i>Charles E. Dohme</i> , Baltimore, Md., <i>Lewis C. Hopp</i>
1899-00	Richmond, Va., May 7, 1900	<i>Albert B. Prescott</i> , Ann Arbor, Mich., <i>T. Ashby Miller</i>
1900-01	St. Louis, Mo., Sept. 16, 1901	<i>Jno. F. Patton</i> , York, Pa., <i>H. M. Whelpley</i>
1901-02	Philadelphia, Pa., Sept. 8, 1902	<i>Henry M. Whelpley</i> , St. Louis, Mo., <i>William L. Cliffe</i>
1902-03	Mackinac Island, Mich., Aug. 3, 1903	<i>Geo. F. Payne</i> , Atlanta, Ga., <i>F. W. R. Perry</i>
1903-04	Kansas City, Mo., Sept. 5, 1904	<i>Lewis C. Hopp</i> , Cleveland, O., <i>Joseph C. Wirthman</i>
1904-05	Atlantic City, N. J., Sept. 4, 1905	<i>James H. Beal</i> , Scio, O., <i>William C. Westcott</i>
1905-06	Indianapolis, Ind., Sept. 3, 1906	<i>Jos. L. Lemberger</i> , Lebanon, Pa., <i>Frank H. Carter</i>
1906-07	New York, N. Y., Sept. 2, 1907	<i>Leo Eliel</i> , South Bend, Ind., <i>Thomas P. Cook</i>
1907-08	Hot Springs, Ark., Sept. 7, 1908	<i>Wm. M. Searby</i> , San Francisco, Cal., <i>Martin A. Eisele</i>
1908-09	Los Angeles, Cal., Aug. 16, 1909	<i>Oscar Oldberg</i> , Chicago, Ill., <i>Thomas W. Jones</i>
1909-10	Richmond, Va., May 2, 1910	<i>Henry H. Rusby</i> , Newark, N. J., <i>T. Ashby Miller</i>
1910-11	Boston, Mass., Aug. 14, 1911	<i>Eugene G. Eberle</i> , Dallas, Tex., <i>C. Herbert Packard</i>
1911-12	Denver, Colo., Aug. 19, 1912	<i>John G. Godding</i> , Boston, Mass., <i>Charles M. Ford</i>
1912-13	Nashville, Tenn., Aug. 18, 1913	<i>William B. Day</i> , Chicago, Ill., <i>James O. Burge</i>
1913-14	Detroit, Mich., Aug. 24, 1914	<i>George M. Beringer</i> , Camden, N. J., <i>Leonard A. Seltzer</i>
1914-15	San Francisco, Cal., Aug. 9, 1915	<i>Caswell A. Mayo</i> , New York, N. Y., <i>John H. Dawson</i>
1915-16	Atlantic City, N. J., Sept. 5, 1916	<i>Wm. C. Alpers</i> , Cleveland, O., <i>Chas. Holzhauser</i>
1916-17	Indianapolis, Ind., Aug. 28, 1917	<i>Fred J. Wulling</i> , Minneapolis, Minn., <i>Francis E. Bibbins</i>
1917-18	Chicago, Ill., Aug. 12, 1918	<i>Charles Holzhauser</i> ,* Newark, N. J., <i>E. N. Gathercoal</i>
1918-19	New York, N. Y., Aug. 25, 1919	<i>Charles H. LaWall</i> , Philadelphia, Pa., <i>Hugo H. Schaefer</i>

PRESIDENTS AND LOCAL SECRETARIES (*Continued*)

Year	Place of Meeting and Date	Presidents and Local Secretaries
1919-20	Washington, D C , May 6, 1920	<i>L E Sayre</i> , Lawrence, Kans , <i>S L Hillon</i>
1920-21	New Orleans, La , Sept 6, 1921	<i>C. Herbert Packard</i> , East Boston, Mass , <i>George W. McDuff</i>
1921-22	Cleveland, O , Aug 14, 1922	<i>Samuel L Hillon</i> , Washington, D C , <i>Edward Spease</i>
1922-23	Ashville, N C , Sept 4, 1923	<i>Juhus A. Koch</i> , Pittsburgh, Pa , <i>John G Beard</i>
1923-24	Buffalo, N Y , Aug 25, 1924	<i>H V Army</i> , New York, N Y , <i>W G Gregory</i>
1924-25	Des Moines, Ia , Aug 24, 1925	<i>C W Holton</i> , Newark, N J , <i>E O Kagy</i>
1925-26	Philadelphia, Pa , Sept 13, 1926	<i>L L Walton</i> , Williamsport, Pa , <i>Ambrose Hunsberger</i>
1926-27	St Louis, Mo , Aug 22, 1927	<i>T. J. Bradley</i> , Boston, Mass , <i>A W Pauley</i>
1927-28	Portland, Me , Aug 20-25, 1928	<i>C W Johnson</i> , Seattle, Wash , <i>E F Carswell</i>
1928-29	Rapid City, S Dak , Aug 26-31, 1929	<i>D F Jones</i> , Watertown, S Dak , <i>Floyd W Brown</i>
1929-30	Baltimore, Md , May 5-10, 1930	<i>H A B Dunning</i> , Baltimore, Md , <i>S Y Harris</i>
1930-31	Miami, Fla , July 27-Aug 1, 1931	<i>H C Christensen</i> , Chicago, Ill , <i>G H Grommet</i>
1931-32	Toronto, Can , Aug 22-27, 1932	<i>Walter D Adams</i> , Forney, Tex , <i>R B J Stanbury</i>
1932-33	Madison, Wis , Aug 26-Sept 1, 1933	<i>W Bruce Philp</i> , Washington, D C , <i>Emerson D Stanley</i>
1933-34	Washington, D C , May 5-12, 1934	<i>Robert L Swain</i> , Baltimore, Md , <i>Frank Delgado</i> , Washington D C
1934-35	Portland, Ore , Aug 5-10, 1935	<i>Robert P Fischelis</i> , Trenton, N J , <i>A O Mickelsen</i> , Portland, Ore
1935-36	Dallas, Tex , Aug 24-29, 1936	<i>P. H Costello</i> , Cooperstown, N Dak , <i>Walter D Adams</i> , Dallas, Tex
1936-37	New York, N Y , Aug 16-21, 1937	<i>George D Beal</i> , Pittsburgh, Pa , <i>Hugo H Schaefer</i> , New York, N Y
1937-38	Minneapolis, Minn , Aug 22-27, 1938	<i>E N Gathercoal</i> , Chicago, Ill , <i>Chas H Rogers</i> , Minneapolis, Minn
1938-39	Atlanta Ga , Aug 20-26, 1939	<i>J Leon Lascoff</i> , New York, N Y , <i>R C Wilson</i> , Atlanta, Ga
1939-40	Richmond, Va , May 7-11, 1940	<i>A G DuMez</i> , Baltimore, Md , <i>L C Bird</i> , Richmond, Va
1940-41	Detroit, Mich , Aug 17-23, 1941	<i>Charles H Evans</i> , Warrenton, Ga , <i>Bernard A Bialk</i> , Detroit, Mich
1941-42	Denver Colo Aug 16-22 1942	<i>B V Christensen</i> , Columbus, O , <i>Paul G Stodghill</i> , Denver, Colo
1942-43	Columbus O Sept 9-11, 1943	<i>Roy Bird Cook</i> , Charleston, W Va , <i>B V Christensen</i> , Columbus O
1943-44	Cleveland O , Sept 7-9, 1944	<i>Ivor Griffith</i> , Philadelphia, Pa , <i>A P Gegenheimer</i> , Cleveland, O
1944-45	No meeting	<i>Geo A Moulton</i> , Peterborough, N H
1945-46	Pittsburgh Pa Aug 27-30 1946	<i>G A Moulton</i> , Peterborough, N H , <i>Stephen Wilson</i> , Pittsburgh Pa
1946-47	Milwaukee Wis Aug 26-30 1947	<i>Earl R Serles</i> , Chicago, Ill , <i>Walter A Koepke</i> , Milwaukee Wis
1947-48	San Francisco, Cal , Aug 8-14, 1948	<i>Sylvester H Dretzka</i> , Milwaukee, Wis , <i>Linnet M Walsh</i> , Burlingame, Calif
1948-49	Jacksonville Fla April 24-30 1949	<i>Ernest Little</i> , Newark N J , <i>J K Attwood</i> , Jacksonville Fla
1949-50	Atlantic City, N J , April 30-May 5 1950	<i>Glenn L Jenkins</i> , Lafayette, Ind , <i>Thomas D Rowe</i> , Newark, N J
1950-51	Buffalo N Y , Aug 26-31, 1951	<i>Henry H Gregg</i> , Minneapolis, Minn , <i>M D Pritchard</i> , Buffalo N Y
1951-52	Philadelphia Pa , Aug 17-23, 1952	<i>Don E Francke</i> , Ann Arbor, Mich , <i>Paul W Wilcox</i> , Philadelphia Pa
1952-53	Salt Lake City, Utah, Aug 16-22 1953	<i>R Q Richards</i> , Fort Myers, Florida, <i>John B Heinz</i> , Salt Lake City, Utah
1953-54	Boston, Mass , August 22-28, 1954	<i>F Royce Franzoni</i> , Washington, D C , <i>Samuel Silverman</i> , Boston, Mass
1954-55	Miami Beach Fla , May 1-6, 1955	<i>N W Stewart</i> , New York, N Y , <i>Jack Davis</i> , Miami Beach Fla
1955-56	Detroit, Mich April 8-13, 1956	<i>John B Heinz</i> , Salt Lake City, Utah, <i>John H Butts</i> , Lansing Mich
1956 57	New York N Y April 28-May 3, 1957	<i>John A MacCartney</i> , Detroit Mich , <i>John L Dandreau</i> New York N Y
1957-58	Los Angeles, Calif April 20-26 1958	<i>Joseph B Burt</i> , Lincoln, Neb , <i>Ben Kingwell</i> , Monrovia, Calif

* Charles Holzhauer died during his term of office and was succeeded by A R L Dohme, the First Vice-President

HONORARY PRESIDENTS

1907-08	<i>Philp C Candius</i> , Mobile, Ala	1920-21	<i>John F. Hancock</i> , Baltimore, Md	1933-34	<i>Edward Kremers</i> , Madison, Wis
1908-09	<i>Samuel A D Sheppard</i> , Boston, Mass	1921-22	<i>John C Wallace</i> , Newcastle, Pa	1934-35	<i>J K Lilly</i> , Indianapolis, Ind
1909-10	<i>Enno Sander</i> , St Louis Mo	1922-23	<i>Thomas D McElhene</i> , Brooklyn N Y	1935-36	<i>D M R Culbreth</i> , Baltimore, Md
1910-11	<i>Ewen McIntyre</i> , New York, N Y.	1923-24	<i>William L DuBois</i> , Catskill N Y	1936-37	<i>W G Gregory</i> , Buffalo, N Y
1911-12	<i>Henry Biroth</i> , Chicago, Ill	1924-25	<i>Louis Emanuel</i> , Pittsburgh, Pa	1937-38	<i>H G Ruenzel</i> , Milwaukee, Wis
1912-13	<i>Thomas F Main</i> , New York, N Y	1925-26	<i>W A Frost</i> , St Paul Minn	1938-39	<i>W C Anderson</i> , Brooklyn, N Y
1913-14	<i>Albert B Lyons</i> , Detroit, Mich	1926-27	<i>W H Rogers</i> , Middletown N Y	1939-40	<i>J W Gayle</i> , Frankfort, Ky
1914-15	<i>Geo H Schaffer</i> , E Madison, Ia	1927-28	<i>Edward Mallinckrodt</i> , St Louis, Mo	1940-41	<i>W P Porterfield</i> , Fargo, N Dak
1915-16	<i>Fabius C Godbold</i> , New Orleans, La	1928-29	<i>F E Stewart</i> , Philadelphia, Pa	1941-42	<i>J C Peacock</i> , Philadelphia, Pa
1916-17	<i>J O Burge</i> , Nashville, Tenn	1929-30	<i>E V Zoeller</i> , Tarkoro, N C	1942-43	<i>James E Hancock</i> , Baltimore, Md
1917-18	<i>W L Dewoody</i> , Pine Bluff, Ark	1930-31	<i>Chie H La Pierre</i> , Cambridge, Mass	1943-44	<i>George Judisch</i> , Ames, Ia
1918-19	<i>Oliver F Fuller</i> , Chicago, Ill	1931-32	<i>Henry S Wellcome</i> , London, England	1944-45	<i>L A Seltzer</i> , Detroit, Mich
1919-20	<i>Alonso B Stevens</i> , Escondido, Cal	1932-33	<i>Charles F Heebner</i> , Toronto, Can	1945-46	<i>L A Seltzer</i> , Detroit, Mich

HONORARY PRESIDENTS (Continued)

1946-47	<i>A C Taylor,</i> Washington, D C	1950-51	<i>Ernest G Eberhardt,</i> Indianapolis Ind	1954-55	<i>E F Cook,</i> Indianapolis Ind
1947-48	<i>Gustavus A Pfeiffer,</i> New York, N Y	1951-52	<i>Carl P Wismer,</i> New York, N Y.	1955-56	<i>Max N Lemberger</i> Milwaukee Wis
1948-49	<i>John Culley</i> Ogden Utah	1952-53	<i>R A Lyman</i> Lincoln Neb	1956-57	<i>I W Nitardy,</i> Brooklyn N Y
1949-50	<i>Robert C Wilson,</i> Athens Ga	1953-54	<i>Elh Lilly,</i> Indianapolis, Ind	1957-58	<i>F O Taylor,</i> S Ft Mitchell Ky

VICE-PRESIDENTS

Year	First Vice-Presidents	Second Vice-Presidents	Third Vice-Presidents
1852-53	<i>George W Andrews,</i> Baltimore, Md	<i>Samuel M Colcord,</i> Boston, Mass	<i>C Augustus Smith,</i> Cincinnati, O
1853-54	<i>George D Coggeshall,</i> New York, N Y	<i>Alexander Duval,</i> Richmond, Va	<i>Charles B Guthrie,</i> Memphis, Tenn
1854-55	<i>Henry T Cummings,</i> Portland, Me	<i>John Meakin,</i> New York, N. Y.	<i>Joseph Laidley,</i> Richmond, Va
1855-56	<i>Charles B Guthrie,</i> Memphis, Tenn	<i>Charles Ellis,</i> Philadelphia, Pa	<i>Henry F Fish,</i> Waterbury, Conn
1856-57	<i>John I Kidwell,</i> Washington, D C	<i>Frederick Stearns,</i> Detroit, Mich	<i>Henry T Kiersted,</i> New York, N Y
1857-58	<i>James Cooke,</i> Frederickshurg, Va	<i>Samuel P Peck,</i> Bennington, Vt	<i>A E Richards,</i> Plaquemine, La
1858-59	<i>Edward R Squibb,</i> Brooklyn, N Y	<i>James O'Gallagher,</i> St Louis, Mo	<i>Robert Bailey,</i> Rome, Ga
1859-60	<i>William Procter, Jr ,</i> Philadelphia, Pa	<i>Joseph Roberts,</i> Baltimore, Md	<i>Edwin O Gale,</i> Chicago, Ill
1860-62	<i>William J M Gordon,</i> Cincinnati, O	<i>William S Thompson,</i> Baltimore, Md	<i>Theodore Metcalf</i> Boston, Mass
1862-63	<i>John Milhau,</i> New York, N Y	<i>Eugene L Massot,</i> St Louis, Mo	<i>J Faris Moore,</i> Baltimore, Md
1863-64	<i>John M Maisch,</i> Philadelphia, Pa	<i>Chas A Tufts,</i> Dover, N H.	<i>George W Weyman</i> Pittsburgh, Pa
1864-65	<i>Richard H. Stabler,</i> Alexandria, Va	<i>Enno Sander,</i> St Louis, Mo	<i>Thomas Hollis,</i> Boston, Mass
1865-66	<i>George C Close,</i> Brooklyn, N Y.	<i>Elijah W Sackrider,</i> Cleveland, O	<i>Charles A Hennish,</i> Lancaster, Pa
1866-67	<i>Edward Parrish,</i> Philadelphia, Pa	<i>Ezekiel H Sargent,</i> Chicago, Ill	<i>John W Shelden,</i> New York, N Y
1867-68	<i>Robert J Brown,</i> Leavenworth Kans	<i>N Hynson Jennings,</i> Baltimore, Md	<i>Daniel Henchman,</i> Boston, Mass
1868-69	<i>Ferris Bringham,</i> Wilmington, Del	<i>Edward S Wayne,</i> Cincinnati, O	<i>Albert E Ebert,</i> Chicago, Ill
1869-70	<i>Ferdinand W Sennewald,</i> St Louis, Mo	<i>John J Pope,</i> New Orleans, La	<i>Joel S Orne,</i> Cambridgeport, Mass
1870-71	<i>Fleming G Greve,</i> Milledgeville, Ga	<i>James G Steele,</i> San Francisco, Cal	<i>Eugene L Massot,</i> St Louis, Mo
1871-72	<i>C Lewis Diehl,</i> Louisville, Ky	<i>George F H Markoe,</i> Boston, Mass	<i>Matthew F Ash,</i> Jackson, Miss
1872-73	<i>Samuel S Garrigues,</i> E Saginaw, Mich	<i>Eduard P Nichols,</i> Newark, N J	<i>Henry C Gaylord,</i> Cleveland, O
1873-74	<i>William Saunders,</i> London, Ont	<i>John T Buck,</i> Jackson, Miss	<i>Paul Balluff,</i> New York, N Y
1874-75	<i>Joseph Roberts,</i> Baltimore, Md	<i>William T Wenzell,</i> San Francisco Cal	<i>Augustus R Bayley,</i> Cambridgeport, Mass
1875-76	<i>Frederick Hoffman,</i> New York, N Y	<i>T Roberts Baker,</i> Richmond, Va	<i>Christian F G Meyer,</i> St Louis, Mo
1876-77	<i>Samuel A D Sheppard,</i> Boston, Mass	<i>Gustavus J. Luhn,</i> Charleston, S C	<i>Jacob D Wells</i> Cincinnati, O
1877-78	<i>Ewen McIntyre,</i> New York, N Y	<i>John Ingalls,</i> Macon, Ga	<i>Emlen Painter,</i> San Francisco, Cal
1878-79	<i>Frederick T Whiting,</i> Great Barrington, Mass	<i>Henry J Rose,</i> Toronto Can	<i>William H Crawford,</i> St Louis, Mo
1879-80	<i>T Roberts Baker,</i> Richmond, Va	<i>Joseph L Lemberger,</i> Lebanon, Pa	<i>Philip C Candisus,</i> Mobile, Ala
1880-81	<i>George H Schafer,</i> Fort Madison, Ia	<i>William S Thompson,</i> Washington, D C	<i>William Simpson,</i> Raleigh, N C
1881-82	<i>Emlen Painter,</i> San Francisco, Cal	<i>George Leis,</i> Lawrence, Kans	<i>John F. Judge,</i> Cincinnati, O
1882-83	<i>John Ingalls,</i> Macon, Ga	<i>Louis Dohme,</i> Baltimore, Md	<i>William B Blanding</i> Providence, R I

VICE-PRESIDENTS (Continued)

Year	First Vice-Presidents	Second Vice-Presidents	Third Vice-Presidents
1883-84	<i>Charles Rice</i> , New York, N Y	<i>Frederick H Mast</i> , Norfolk, Va	<i>Edward W Runyon</i> , San Francisco, Cal
1884-85	<i>John A Dadd</i> , Milwaukee, Wis	<i>Henry Canning</i> , Boston, Mass	<i>Charles F Goodman</i> , Omaha, Nebr
1885-86	<i>Albert H Hollister</i> , Madison, Wis	<i>Albert B Prescott</i> , Ann Arbor, Mich	<i>Joseph S Evans</i> , West Chester, Pa
1886-87	<i>Henry J Menninger</i> , Brooklyn, N Y	<i>M W Alexander</i> , St Louis, Mo	<i>Norman A. Kuhn</i> , Omaha, Nebr
1887-88	<i>M W Alexander</i> , St Louis, Mo	<i>A K Finlay</i> , New Orleans, La	<i>Karl Simmon</i> , St Paul, Minn
1888-89	<i>James Vernor</i> , Detroit, Mich	<i>Fred Wilcox</i> , Waterbury, Conn	<i>Alvin A Yeager</i> , Knoxville, Tenn
1889-90	<i>Karl Simmon</i> , St Paul Minn	<i>Wm M Searby</i> , San Francisco Cal	<i>Joseph W Eckford</i> , Aberdeen, Miss
1890-91	<i>A B Stevens</i> , Ann Arbor, Mich	<i>Chas E Dohme</i> , Baltimore, Md	<i>James M. Good</i> , St Louis, Mo
1891-92	<i>Geo J Seabury</i> , New York, N Y	<i>W H Torbert</i> , Dubuque, Ia	<i>L T Dunning</i> , Sioux Falls, S Dak
1892-93	<i>A P Preston</i> , Portsmouth, N H	<i>Sidney P Watson</i> , Atlanta, Ga	<i>Wm H Averill</i> , Frankfort, Ky
1893-94	<i>Leo Etel</i> , South Bend Ind	<i>Wiley Rogers</i> , Louisville, Ky	<i>Chas Caspari, Jr</i> , Baltimore, Md
1894-95	<i>Chas M Ford</i> , Denver, Colo	<i>Jno N Hurly</i> , Indianapolis, Ind	<i>Jas E Morrison</i> , Montreal, Can
1895-96	<i>Chas E Dohme</i> , Baltimore, Md	<i>A Brandenburger</i> , Jefferson City, Mo	<i>Mrs M O Miner</i> , Hiawatha, Kans
1896-97	<i>Geo F Payne</i> , Atlanta Ga	<i>Wm A Frost</i> , St Paul, Minn	<i>Geo W Parisen</i> , Perth Amboy, N J
1897-98	<i>George C Bartells</i> , Camp Point, Ill	<i>Wm S Thompson</i> , Washington, D C	<i>Jacob A Miller</i> , Harrisburg, Pa
1898-99	<i>George F Payne</i> , Atlanta Ga	<i>James H Beal</i> , Scio, O	<i>Josie A Wanous</i> , Minneapolis, Minn
1899-00	<i>Lewis C Hopp</i> , Cleveland, O	<i>Wm L Dewooddy</i> , Pine Bluff, Ark	<i>Henry R Gray</i> , Montreal, Can
1900-01	<i>James H Beal</i> , Scio O	<i>Jno W Gayle</i> , Frankfort, Ky	<i>E A Ruddiman</i> , Nashville, Tenn
1901-02	<i>Wm M Searby</i> , San Francisco Cal	<i>George F Poyne</i> , Atlanta, Ga	<i>Wm S Thompson</i> , Washington, D C
1902-03	<i>Wm L Cliffe</i> , Philadelphia Pa	<i>Eugene G Eberle</i> , Dallas, Tex	<i>Henry Willis</i> , Quebec, Can
1903-04	<i>Wm C Alpers</i> , New York, N Y	<i>Albert M Roehrig</i> , Stapleton, N Y	<i>Otto F Claus</i> , St Louis, Mo
1904-05	<i>Philip C Candidus</i> , Mobile, Ala	<i>Wm Mittelbach</i> , Boonville, Mo	<i>Julius A Koch</i> , Pittsburgh, Pa
1905-06	<i>Chas Holzhauser</i> , Newark, N J	<i>Chas A Rapelye</i> , Hartford, Conn	<i>Gabius C Godbold</i> , New Orleans, La
1906-07	<i>Wm Mittelbach</i> , Boonville, Mo	<i>C S N Hallberg</i> , Chicago, Ill	<i>Thomas P Cook</i> , New York, N Y
1907-08	<i>Oscar Oldberg</i> , Chicago, Ill	<i>Henry H Rusby</i> , New York, N Y	<i>Oscar W Belhea</i> , Meridian, Miss
1908-09	<i>Eugene G Eberle</i> , Dallas, Tex.	<i>Wm Mittelbach</i> , Boonville, Mo	<i>James H Beal</i> , Scio, O
1909-10	<i>Clement B Lowe</i> , Philadelphia, Pa	<i>Chas W Johnson</i> , Seattle, Wash	<i>Wm B Day</i> , Chicago Ill
1910-11	<i>Wm B Day</i> , Chicago, Ill	<i>Otto F Claus</i> , St Louis, Mo	<i>Leonard A Seltzer</i> , Detroit, Mich
1911-12	<i>W Bodemann</i> , Chicago, Ill	<i>Chas M Ford</i> , Denver, Colo	<i>Ernest Berger</i> , Tampa, Fla
1912-13	<i>Chas M Ford</i> , Denver Colo	<i>Caswell A Moyo</i> , New York, N Y	<i>C Herbert Packard</i> , East Boston Mass
1913-14	<i>Franklin M Apple</i> , Philadelphia, Pa	<i>Wm S. Richardson</i> , Washington, D C	<i>L D Havenhill</i> , Lawrence, Kans
1914-15	<i>L D Havenhill</i> , Lawrence, Kans	<i>C Herbert Packard</i> , East Boston, Mass	<i>Charles Gietner</i> , St Louis, Mo
1915-16	<i>C H LaWall</i> , Philadelphia, Pa	<i>E A Ruddiman</i> , Nashville, Tenn	<i>Inwood A Brown</i> , Lexington Ky
1916-17	<i>Leonard A Seltzer</i> , Detroit, Mich	<i>Lucius E Soyre</i> , Lawrence, Kans	<i>Philip Asher</i> , New Orleans, La
1917-18	<i>Alfred R L Dohme</i> , Baltimore, Md	<i>Leonard A Seltzer</i> , Detroit, Mich	<i>Theodore J Bradley</i> , Boston Mass
1918-19	<i>F W Nitardy</i> , Brooklyn, N Y.	<i>Theodore J Bradley</i> , Boston, Mass	<i>Francis Hemm</i> , St Louis, Mo

VICE-PRESIDENTS (Continued)

Year	First Vice-Presidents	Second Vice-Presidents	Third Vice-Presidents
1919-20	<i>Theo. J. Bradley</i> , Boston, Mass.	Harry Whitehouse, Johnson City, Tenn.	E. Fullerton Cook, Philadelphia, Pa.
1920-21	E. Fullerton Cook, Philadelphia, Pa.	<i>Charles E. Caspari</i> , St. Louis, Mo.	<i>W. P. Parterfield</i> , Fargo, N. D.
1921-22	<i>Charles E. Caspari</i> , St. Louis, Mo.	<i>David F. Jones</i> , Watertown, S. D.	H. H. Schaefer, New York, N. Y.
1922-23	<i>E. N. Gathercoal</i> , Chicago, Ill.	<i>Lyman F. Kebler</i> , Washington, D. C.	Clyde L. Eddy, New York, N. Y.
1923-24	<i>L. F. Kebler</i> , Washington, D. C.	<i>F. E. Bibbins</i> , Indianapolis, Ind.	<i>W. Bruce Philip</i> , San Francisco, Calif.
1924-25	Paul S. Pittenger, Philadelphia, Pa.	William Mansfield, Delmar, N. Y.	
1925-26	<i>W. C. Anderson</i> , Brooklyn, N. Y.	C. L. Eddy, New York, N. Y.	
1926-27	George Judisch, Ames, Ia.	<i>A. G. Hulett</i> , Phoenix, Ariz.	
1927-28	<i>Ambrose Hunsberger</i> , Philadelphia, Pa.	<i>Joseph Jacobs</i> , Atlanta, Ga.	
1928-29	<i>A. W. Pauley</i> , St. Louis, Mo.	<i>W. H. Zeigler</i> , Charleston, S. C.	
1929-30	<i>A. L. I. Winne</i> , Richmond, Va.	W. B. Goodyear, Harrisburg, Pa.	
1930-31	Walter D. Adams, Forney, Tex.	<i>D. B. R. Johnson</i> , Norman, Okla.	
1931-32	<i>J. G. Beard</i> , Chapel Hill, N. C.	John W. Dargavel, Minneapolis, Minn.	
1932-33	Rowland Jones, Jr. Gettysburg, S. Dak.	G. H. Grommet, Miami, Fla.	
1933-34	Robert P. Fischelis, Trenton, N. J.	J. C. Krantz, Jr., Baltimore, Md.	
1934-35	Geo. D. Beal, Pittsburgh, Pa.	Oscar Rennehohm, Madison, Wis.	
1935-36	F. A. Delgado, Washington, D. C.	J. Lester Hayman, Morgantown, W. Va.	
1936-37	<i>J. Leon Lascaff</i> , New York, N. Y.	James C. Munch, Lansdowne, Pa.	
1937-38	W. Mac Childs, Eldorado, Kans.	Glenn L. Jenkins, Minneapolis, Minn.	
1938-39	<i>A. O. Mickelsen</i> , Portland, Ore.	Geo. A. Moulton, Peterborough, N. H.	
1939-40	F. O. Taylor, Detroit, Mich.	<i>F. J. Cermak</i> , Cleveland, O.	
1940-41	<i>H. A. K. Whitney</i> , Ann Arbor, Mich.	Henry H. Gregg, Minneapolis, Minn.	
1941-42	J. K. Attwood, Jacksonville, Fla.	L. W. Rowe, Detroit, Mich.	
1942-43	Donald A. Clarke, New York, N. Y.	C. O. Lee, Lafayette, Ind.	
1943-44	P. G. Stodghill, Denver, Colo.	<i>J. G. Beard</i> , Chapel Hill, N. C.	
1944-46	<i>C. E. Wilsan</i> , Corinth, Miss.	<i>R. S. Lehman</i> , Brooklyn, N. Y.	
1946-47	<i>A. Lee Adams</i> , Glencoe, Ill.	H. V. Darnell, Indianapolis, Ind.	
1947-48	Augustus J. Affleck, Sacramento, Calif.	<i>Roy L. Sanford</i> , Enid, Okla.	
1948-49	Mearl D. Pritchard, Buffalo, N. Y.	Frederick D. Lascoff, New York, N. Y.	
1949-50	Harold C. Kinner, Washington, D. C.	Leih L. Riggs, Portland, Ore.	
1950-51	Roy A. Bowers, Albuquerque, N. M.	Louis J. Fischl, Oakland, Calif.	
1951-52	Joseph B. Burt, Lincoln, Nehr.	John A. MacCartney, Detroit, Mich.	
1952-53	Tom Rowe, Ann Arbor, Mich.	Charles F. Lanwermyer, Waukegan, Ill.	
1953-54	John A. MacCartney, Detroit, Mich.	Joseph B. Sprowls, Philadelphia, Pa.	
1954-55	John B. Heinz, Salt Lake City, Utah	Ronald V. Robertson, Spokane, Wash.	
1955-56	Troy C. Daniels, San Francisco, Calif.	George C. Roberts, Greenwood, Miss.	
1956-57	R. V. Robertson, Spokane, Wash.	John J. Dugan, New Haven, Conn.	
1957-58	J. W. Lansdowne, Indianapolis, Ind.	Leroy A. Weidle, Sr., St. Louis, Mo.	

LIST OF OFFICERS (*Continued*)

TREASURERS

1852-54	<i>Alfred B Taylor,</i> Philadelphia, Pa	1860-63	<i>Henry Haviland,</i> New York, N Y	1908-21	<i>Henry M Whelpley</i> St Louis, Mo
1854-56	{ <i>Somuel L Colcord,</i> Boston, Mass	1863-65	<i>J Brown Boxley,</i> Baltimore, Md	1921-25	<i>E F Kelly,</i> Baltimore, Md
1857-59		1865-86	<i>Charles A Tufis,</i> Dover, N H	1925-41	<i>C. W Holton,</i> Essex Fells N J
1856-57	<i>James S Aspinwall,</i> New York, N Y	1886-1908	<i>S A D Sheppard,</i> Boston, Mass	1941-	<i>Hugo H Schaefer,</i> Brooklyn, N Y
1859-60	<i>Ashel Boyden,</i> Boston Mass				

SECRETARIES

Recording Secretaries		Corresponding Secretaries		General Secretaries	
1855-59	<i>William J M Gordon,</i> Cincinnati, O	1852-53	{ <i>William Procter, Jr</i> Philadelphia, Pa	1863-93	<i>John M Maassch,</i> Philadelphia, Pa
1859-60	<i>Charles Bullock,</i> Philadelphia, Pa.	1854-57		1893	<i>Henry M Whelpley,</i> St Louis (Acting)
1860-62	<i>James T Shinn,</i> Philadelphia Pa.	1853-54	<i>William B Chapman</i> Cincinnati, O	1893-94	<i>Joseph P Remington,</i> Philadelphia, Pa
1862-63	<i>Peter W Bedford</i> New York, N Y.	1857-58	<i>Edward Parrish,</i> Philadelphia, Pa	1894 1911	<i>Chas Caspari, Jr</i> Baltimore, Md
1863-64	<i>William Evans, Sr</i> Philadelphia, Pa	1858-59	<i>Ambrose Smith,</i> Philadelphia, Pa	1911-14	<i>James H Beal,</i> Scioto, O
1864-65	<i>Henry N Rittenhouse</i> Philadelphia Pa	1859-60	<i>William Hegeman,</i> New York, N Y	1914-25	<i>William B Day,</i> Chicago, Ill
		1860-62	{ <i>Peter W Bedford</i> New York, N Y	1925-44	<i>E F Kelly</i> Washington D C
		1863-65		1945-	<i>R P Fischels,</i> Washington D C
		1862-63	<i>John M. Maassch,</i> Philadelphia, Pa		

EDITORS OF THE JOURNAL

Scientific Edition

1912-14	<i>J H Beol</i> Scioto O	1940-42	<i>A G DuMez,</i> Baltimore, Md
1914-15	<i>E C Marshall</i> Columbus O	1942-	<i>J L Powers,</i> Washington, D C
1915-40	<i>E G Eberle</i> Washington D C		

Practical Pharmacy Edition

1940-41	<i>E F Kelly,</i> Washington, D C
1941-43	<i>R W Rodman,</i> Washington, D C
1943-48	<i>Glenn Sonnedecker,</i> Washington, D C
1948-55	<i>R P Fischels,</i> Washington D C
1956-	<i>E W Martin,</i> Washington D C

OFFICERS OF THE COUNCIL

Year	Chairmen	Vice-Chairmen	Secretaries
1880-81	<i>Jos P Remington</i>	<i>Joseph Roberts</i>	<i>George W Kennedy</i>
1881-83	<i>Jos P Remington</i>	<i>Wm J M Gordon</i>	<i>George W Kennedy</i>
1883-84	<i>Jos P Remington</i>	<i>C Lewis Diehl</i>	<i>George W Kennedy</i>
1884-85	<i>Jos P Remington</i>	<i>John A Dadd</i>	<i>George W Kennedy</i>
1885-86	<i>Jos P Remington</i>	<i>C Lewis Diehl</i>	<i>George W Kennedy</i>
1886-87	<i>Wm S Thompson</i>	<i>H J Menninger</i>	<i>George W Kennedy</i>
1887-88	<i>Wm H Rogers</i>	<i>Karl Simmon</i>	<i>George W Kennedy</i>
1888-89	<i>Jas M Good</i>	<i>Emlen Painter</i>	<i>George W Kennedy</i>
1889-92	<i>Jas M Good</i>	<i>Wm S Thompson</i>	<i>George W Kennedy</i>
1892-94	<i>Jas M Good</i>	<i>H M Whitney</i>	<i>George W Kennedy</i>
1894-95	<i>Wm S Thompson</i>	<i>H M Whitney</i>	<i>George W Kennedy</i>
1895-96	<i>Wm S Thompson</i>	<i>Wm C Alpers</i>	<i>George W Kennedy</i>
1896-01	<i>Wm S Thompson</i>	<i>Jas M Good</i>	<i>George W Kennedy</i>
1901-02	<i>A B Prescott</i>	<i>Chas E Dohme</i>	<i>George W Kennedy</i>
1902-03	<i>James H Beal</i>	<i>Lewis C Hopp</i>	<i>Henry M Whelpley</i>
1903-04	<i>James H Beal</i>	<i>Leo Ehel</i>	<i>Henry M Whelpley</i>
1904-05	<i>James H Beal</i>	<i>Jos L Lemberger</i>	<i>Henry M Whelpley</i>
1905-06	<i>James H Beal</i>	<i>Wm C Alpers</i>	<i>Henry M Whelpley</i>
1906-08	<i>James H Beal</i>	<i>Albert M Roehrig</i>	<i>Henry M Whelpley</i>
1908-09	<i>Jos P Remington</i>	<i>Wm S Searby</i>	<i>Joseph W England</i>
1909-10	<i>Fabius C Godbold</i>	<i>Julius A Koch</i>	<i>Joseph W England</i>
1910-11	<i>James H Beal</i>	<i>Henry H Rusby</i>	<i>Joseph W England</i>
1911-12	<i>Eugene G Eberle</i>	<i>James M Good</i>	<i>Joseph W England</i>
1912-13	<i>Eugene G Eberle</i>	<i>Fabius C Godbold</i>	<i>Joseph W England</i>
1913-16	<i>Eugene G Eberle</i>	<i>J G Godding</i>	<i>Joseph W England</i>
1916-19	<i>Lewis C Hopp</i>	<i>S L Hilton</i>	<i>Joseph W England</i>
1919-20	<i>Lewis C Hopp</i>	<i>Charles H LaWall</i>	<i>Joseph W England</i>
1920-22	<i>Charles H LaWall</i>	<i>Charles E Caspari</i>	<i>A G DuMes</i>
1922-23	<i>S L Hilton</i>	<i>Charles E Caspari</i>	<i>A G DuMes</i>
1923-24	<i>James H Beal</i>	<i>Charles E Caspari</i>	<i>R P Fischelis</i>
1924-25	<i>James H Beal</i>	<i>S L Hilton</i>	<i>R P Fischelis</i>
1925-26	<i>S L Hilton</i>	<i>W C Anderson</i>	<i>E F Kelly</i>
1926-34	<i>S L Hilton</i>	<i>C H LaWall</i>	<i>E F Kelly</i>
1934-35	<i>S L Hilton</i>	<i>J H Beal</i>	<i>E F Kelly</i>
1935-37	<i>S L Hilton</i>	<i>H C Christensen</i>	<i>E F Kelly</i>
1937-38	<i>S L Hilton</i>	<i>George D Beal</i>	<i>E F Kelly</i>
1938-39	<i>S L Hilton</i>	<i>Glenn L Jenkins</i>	<i>E F Kelly</i>
1939-40	<i>S L Hilton</i>	<i>Glenn L Jenkins</i>	<i>E F Kelly</i>
1940-41	<i>Glenn L Jenkins</i>	<i>R P Fischelis</i>	<i>E F Kelly</i>
1941-42	<i>R P Fischelis</i>	<i>P H Costello</i>	<i>E F Kelly</i>
1942-43	<i>R P Fischelis</i>	<i>C H Evans</i>	<i>E F Kelly</i>
1943-45	<i>R P Fischelis</i>	<i>C H Evans</i>	<i>E F Kelly</i>
1945-46	<i>George D Beal</i>	<i>C H Evans</i>	<i>R P Fischelis</i>
1946-47	<i>George D Beal</i>	<i>Henry H Gregg</i>	<i>R P Fischelis</i>
1947-48	<i>George D Beal</i>	<i>George A Moulton</i>	<i>R P Fischelis</i>
1948-49	<i>George D Beal</i>	<i>George A Moulton</i>	<i>R P Fischelis</i>
1949-50	<i>George D Beal</i>	<i>George A Moulton</i>	<i>R P Fischelis</i>
1950-51	<i>George D Beal</i>	<i>Roy L Sanford</i>	<i>R P Fischelis</i>
1951-52	<i>George D Beal</i>	<i>Roy L Sanford</i>	<i>R P Fischelis</i>
1952-53	<i>Ernest Little</i>	<i>Glenn L Jenkins</i>	<i>R P Fischelis</i>
1953-54	<i>Glenn L Jenkins</i>	<i>George A Moulton</i>	<i>R P Fischelis</i>
1954-55	<i>Glenn L Jenkins</i>	<i>George A Moulton</i>	<i>R P Fischelis</i>
1955-56	<i>R Q Richards</i>	<i>Henry H Gregg</i>	<i>R P Fischelis</i>
1956-57	<i>J B Heinz</i>	<i>Henry H Gregg</i>	<i>R P Fischelis</i>
1957-58	<i>J B Heinz</i>	<i>Henry H Gregg</i>	<i>R P Fischelis</i>

OFFICERS OF THE HOUSE OF DELEGATES

Year	Chairmen	1st Vice-Chairmen	2nd Vice-Chairmen	Secretaries
1912-13	<i>W C Anderson</i>	<i>C M Snow</i>	<i>H S Richardson</i>	<i>Clarissa M Roehr</i>
1913-14	<i>C M Snow</i>	<i>W S Richardson</i>	<i>O F Claus</i>	<i>R A Kuever</i>
1914-15	<i>W S Richardson</i>	<i>C B Jordan</i>	<i>H M Faser</i>	<i>Joseph Weinstein</i>
1915-16	<i>H P Hynson</i>	<i>F W Nivardy</i>	<i>O F Claus</i>	<i>Jeannot Hostmann</i>
1916-17	<i>I H Beal</i>	<i>S C Henry</i>	<i>O F Claus</i>	<i>Jeannot Hostmann</i>
1917-18	<i>S C Henry</i>	<i>O F Claus</i>	<i>S L Hilton</i>	<i>Jeannot Hostmann</i>
1918-19	<i>O F Claus</i>	<i>S L Hilton</i>	<i>E F Kelly</i>	<i>Jeannot Hostmann</i>
1919-20	<i>S L Hilton</i>	<i>E F Kelly</i>	<i>E L Neucomb</i>	<i>Jeannot Hostmann</i>
1920-21	<i>E F Kelly</i>	<i>J G Beard</i>	<i>Adam Wirth</i>	<i>Jeannot Hostmann</i>
1921-22	<i>E F Kelly</i>	<i>E L Neucomb</i>	<i>W B Philip</i>	<i>Jeannot Hostmann</i>
1922-23	<i>E F Kelly</i>	<i>E L Neucomb</i>	<i>W B Philip</i>	<i>Wm B Day</i>
1923-24	<i>L L Walton</i>	<i>W Bruce Philip</i>		<i>Wm B Day</i>
1924-25	<i>W Bruce Philip</i>	<i>W D Jones</i>		<i>Wm B Day</i>
1925-26	<i>W D Jones</i>	<i>Jacob Diner</i>		<i>E F Kelly</i>

OFFICERS OF THE HOUSE OF DELEGATES (*Continued*)

Year	Chairmen	1st Vice-Chairmen	2nd Vice-Chairmen	Secretaries
1926-27	<i>Jacob Diner</i>	<i>L A Seltzer</i>		<i>E F. Kelly</i>
1927-28	<i>L A Seltzer</i>	<i>Ambrose Hunsberger</i>		<i>E F. Kelly</i>
1928-29	<i>Ambrose Hunsberger</i>	<i>R L Swam</i>		<i>E F. Kelly</i>
1929-30	<i>R L Swam</i>	<i>C B Jordan</i>		<i>E F. Kelly</i>
1930-31	<i>C B Jordan</i>	<i>Thomas Roach</i>		<i>E F. Kelly</i>
1931-32	<i>Thomas Roach</i>	<i>J W Slocum</i>		<i>E F. Kelly</i>
1932-33	<i>J W Slocum</i>	<i>P H Costello</i>		<i>E F. Kelly</i>
1933-34	<i>P H Costello</i>	<i>S A Williams</i>		<i>E F. Kelly</i>
1934-35	<i>Rowland Jones, Jr</i>	<i>S A Williams</i>		<i>E F. Kelly</i>
1935-36	<i>Roy B Cook</i>	<i>C Thurston Gilbert</i>		<i>E F. Kelly</i>
1936-37	<i>Robert C Wilson</i>	<i>A F Ludwig</i>		<i>E F. Kelly</i>
1937-38	<i>A L I Winne</i>	<i>Ernest Little</i>		<i>E F. Kelly</i>
1938-39	<i>C H Rogers</i>	<i>R A Kuever</i>		<i>E F. Kelly</i>
1939-40	<i>M N. Ford</i>	<i>E C Severin</i>		<i>E F. Kelly</i>
1940-41	<i>Hugo H Schaefer</i>	<i>Charles L Guthrie</i>		<i>E F. Kelly</i>
1941-42	<i>H H Gregg</i>	<i>C L O'Connell</i>		<i>E F. Kelly</i>
1942-43	<i>J K Attwood</i>	<i>Glenn L Jenkins</i>		<i>E F. Kelly</i>
1943-44	<i>Glenn L Jenkins</i>	<i>S H Dretzka</i>		<i>E F. Kelly</i>
1944-45	<i>S H Dretzka</i>	<i>E L Hammond</i>		<i>E F. Kelly</i>
1945-46	<i>S H Dretzka</i>	<i>E L Hammond</i>		<i>R. P. Fischelis</i>
1946-47	<i>H C Muldoon</i>	<i>E M Josey</i>		<i>R. P. Fischelis</i>
1947-48	<i>Charles H Evans</i>	<i>Emil C Horn</i>		<i>R. P. Fischelis</i>
1948-49	<i>Bert Mull</i>	<i>Louis Fischl</i>		<i>R. P. Fischelis</i>
1949-50	<i>R Q Richards</i>	<i>Newell W Stewart</i>		<i>R. P. Fischelis</i>
1950-51	<i>Newell W Stewart</i>	<i>Thomas D Wyatt</i>		<i>R. P. Fischelis</i>
1951-52	<i>Louis J Fischl</i>	<i>Mearl D Pritchard</i>		<i>R. P. Fischelis</i>
1952-53	<i>E M Josey</i>	<i>Paul W Wilcox</i>		<i>R. P. Fischelis</i>
1953-54	<i>Lesh L Riggs</i>	<i>Louis C Zopf</i>		<i>R. P. Fischelis</i>
1954-55	<i>Thomas D Wyatt</i>	<i>James J Lynch</i>		<i>R. P. Fischelis</i>
1955-56	<i>James J Lynch</i>	<i>W B Shangraw</i>		<i>R. P. Fischelis</i>
1956-57	<i>Troy C Daniels</i>	<i>John H Butts</i>		<i>R. P. Fischelis</i>
1957-58	<i>Nicholas Gesoalde</i>	<i>Ewart A Swinyard</i>		<i>R. P. Fischelis</i>

OFFICERS OF THE SECTIONS

SCIENTIFIC SECTION

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1887-88	<i>T Robert Baker</i>	<i>A B Lyons</i>	1921-22	<i>H. W. Youngken</i>	<i>Arno Viehoever</i>
1888-89	<i>Emlen Pasnter</i>	<i>H M Whelpley</i>	1922-23	<i>Arno Viehoever</i>	<i>J. P. Snyder</i>
1889-90	<i>Henry M Whelpley</i>	<i>C F Dare</i>	1923-24	<i>J P Snyder</i>	<i>Paul S Pittenger</i>
1890-91	<i>E L Patch</i>	<i>C S N Hallberg</i>	1924-25	<i>Paul S Pittenger</i>	<i>F. F. Berg</i>
1891-92	<i>C S N Hallberg</i>	<i>H W Snow</i>	1925-26	<i>F. F. Berg</i>	<i>Paul S Pittenger</i>
1892-93	<i>C T P Fennel</i>	<i>F G Ryan</i>	1926-27	<i>J. C Krantz</i>	<i>Paul S Pittenger</i>
1893-94	<i>L E Sayre</i>	<i>C M Ford</i>	1927-28	<i>L. W. Rowe</i>	<i>Paul S Pittenger</i>
1894-95	<i>A R L Dohme</i>	<i>G B Kauffman</i>	1928-29	<i>J C Munch</i>	<i>L. W. Rowe</i>
1895-96	<i>S P Sadler</i>	<i>W C Alpers</i>	1929-30	<i>H A. Langenhan</i>	<i>L. W. Rowe</i>
1896-97	<i>W C Alpers</i>	<i>V Coblenitz</i>	1930-31	<i>E E. Swanson</i>	<i>L. W. Rowe</i>
1897-98	<i>Edward Kremers</i>	<i>A B Lyons</i>	1931-32	<i>L E. Warren</i>	<i>L. W. Rowe</i>
1898-99	<i>Henry H. Rusby</i>	<i>H V Arny</i>	1932-33	<i>W J. Husa</i>	<i>L. W. Rowe</i>
1899-00	<i>Frank G Ryan</i>	<i>Caswell A Mayo</i>	1933-34	<i>F E. Bibbins</i>	<i>L. W. Rowe</i>
1900-01	<i>Oscar Oldberg</i>	<i>Lyman F Kebler</i>	1934-35	<i>E. V. Lynn</i>	<i>F. E. Bibbins</i>
1901-02	<i>Lyman F Kebler</i>	<i>Jos W England</i>	1935-36	<i>H. M. Burlinge</i>	<i>F. E. Bibbins</i>
1902-03	<i>J O Schlotterbeck</i>	<i>Jos W England</i>	1936-37	<i>Glenn L Jenkins</i>	<i>F. E. Bibbins</i>
1903-04	<i>W A Puckner</i>	<i>Eustace H Gane</i>	1937-38	<i>B V Christensen</i>	<i>F. E. Bibbins</i>
1904-05	<i>Eustace H Gane</i>	<i>C E Caspari</i>	1938-39	<i>C F Lanweremeyer</i>	<i>F. E. Bibbins</i>
1905-06	<i>C. E. Caspari</i>	<i>Daniel Base</i>	1939-40	<i>J B. Burt</i>	<i>F. E. Bibbins</i>
1906-07	<i>Reid Hunt</i>	<i>Virgil Coblenitz</i>	1940-41	<i>J. M. Dille</i>	<i>F. E. Bibbins</i>
1907-08	<i>Virgil Coblenitz</i>	<i>Chas E Vanderkleed</i>	1941-42	<i>W. H. Hartung</i>	<i>F. E. Bibbins</i>
1908-09	<i>Chas E Vanderkleed</i>	<i>Martin I Wilbert</i>	1942-44	<i>Charles O. Wilson</i>	<i>G. L. Bibbins</i>
1909-10	<i>Martin I. Wilbert</i>	<i>Albert H Clark</i>	1945-46	<i>L. W. Hazleton</i>	<i>Ray S Kelley</i>
1910-11	<i>Albert H. Clark</i>	<i>W O. Richtmann</i>	1946-47	<i>L. C. Zopf</i>	<i>Ray S Kelley</i>
1911-12	<i>W. O. Richtmann</i>	<i>C. H. LaWall</i>	1947-48	<i>R. E. Anderson</i>	<i>Ray S Kelley</i>
1912-13	<i>Frank R Eldred</i>	<i>Freeman P Stroup</i>	1948-49	<i>Paul J. Jannke</i>	<i>Ray S Kelley</i>
1913-14	<i>E A. Ruddsman</i>	<i>Wilbur L Scotille</i>	1949-50	<i>Raymond P Ahlquist</i>	<i>Ray S Kelley</i>
1914-15	<i>H. Engelhardt</i>	<i>William Mansfield</i>	1950-51	<i>Earl P. Guth</i>	<i>Ray S Kelley</i>
1915-16	<i>W. L. Scotille</i>	<i>E L Newcomb</i>	1951-52	<i>Lloyd M. Parks</i>	<i>Ray S Kelley</i>
1916-17	<i>J. L. Turner</i>	<i>W W Stockberger</i>	1952-53	<i>Ole Gisvold</i>	<i>Ray S Kelley</i>
1917-18	<i>W W. Stockberger</i>	<i>H C. Fuller</i>	1953-54	<i>Leroy D Edwards</i>	<i>Ray S Kelley*</i>
1918-19	<i>E N Gathercaal</i>	<i>A G DuMez</i>			<i>A J McBay</i>
1919-20	<i>Jacob Diner</i>	<i>A G DuMez</i>	1954-55	<i>Heber W Youngken</i>	<i>A J McBay</i>
1920-21	<i>A G DuMez</i>	<i>H W Youngken</i>	1955-56	<i>Rudolph H Blythe</i>	<i>A J McBay</i>
			1956-57	<i>G P Hager</i>	<i>A J McBay</i>
			1957-58	<i>E A Swinyard</i>	<i>R C Anderson</i>

SECTION ON PRACTICAL PHARMACY AND DISPENSING

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1900-01	<i>H. P. Hynson</i>	<i>F. W. E. Stedem</i>	1921-22	<i>Ivar Griffith</i>	<i>I. A. Becker</i>
1901-02	<i>F. W. E. Stedem</i>	<i>Wm. F. Kaenmerer</i>	1922-23	<i>C. B. Washburn</i>	<i>Robert J. Ruth</i>
1902-03	<i>Gea. M. Beringer</i>	<i>William H. Burke</i>	1923-24	<i>R. J. Ruth</i>	<i>Wm. H. Fard</i>
1903-04	<i>William H. Burke</i>	<i>E. A. Ruddiman</i>	1924-25	<i>John C. Krantz, Jr.</i>	<i>F. J. Blumenschein</i>
1904-05	<i>Charles A. Ropelye</i>	<i>Wm. C. Kirchgessner</i>	1925-26	<i>H. C. Newton</i>	<i>Gustav Bachman</i>
1905-06	<i>Wm. C. Alpers</i>	<i>H. A. B. Dunning</i>	1926-27	<i>A. B. Nichols</i>	<i>C. V. Netz</i>
1906-07	<i>H. A. B. Dunning</i>	<i>Joseph Weinstein</i>	1927-28	<i>A. B. Nichols</i>	<i>P. H. Dirstline</i>
1907-08	<i>Franklin M. Apple</i>	<i>Joseph Weinstein</i>	1928-29	<i>P. H. Dirstline</i>	<i>H. C. Newton</i>
1908-09	<i>L. A. Seltzer</i>	<i>E. F. Cook</i>	1929-30	<i>H. C. Newton</i>	<i>E. O. Leonard</i>
1909-10	<i>O. Raubenheimer</i>	<i>Erich H. Ladish</i>	1930-31	<i>Ralph E. Terry</i>	<i>W. Paul Briggs</i>
1910-11	<i>Louis Saalbach</i>	<i>P. Henry Utech</i>	1931-32	<i>W. G. Crackell</i>	<i>R. E. Terry</i>
1911-12	<i>P. Henry Utech</i>	<i>J. Leon Lascoff</i>	1932-33	<i>W. Paul Briggs</i>	<i>R. E. Terry</i>
1912-13	<i>J. Leon Lascoff</i>	<i>F. W. Nitardy</i>	1933-34	<i>M. J. Andrews</i>	<i>R. E. Terry</i>
1913-14	<i>F. W. Nitardy</i>	<i>Cornelius Osseward</i>	1934-35	<i>H. M. Burlage</i>	<i>L. W. Richards</i>
1914-15	<i>Cornelius Osseward</i>	<i>I. A. Becker</i>	1935-36	<i>L. W. Rising</i>	<i>L. W. Richards</i>
1915-16	<i>Joseph Weinstein</i>	<i>H. B. SeCheverell</i>	1936-37	<i>H. A. K. Whitney</i>	<i>L. W. Richards</i>
1916-17	<i>W. H. Glaver</i>	<i>David Stolz</i>	1937-38	<i>W. J. Husa</i>	<i>Louis C. Zopf</i>
1917-18	<i>Javiah C. Peacock</i>	<i>R. W. Terry</i>	1938-39	<i>L. W. Richards</i>	<i>Louis C. Zopf</i>
1918-19	<i>R. W. Terry</i>	<i>Edward Davy</i>	1939-40	<i>R. W. Clark</i>	<i>Louis C. Zopf</i>
1919-20	<i>E. A. Ruddiman</i>	<i>Ivar Griffith</i>	1940-41	<i>Louis C. Zopf</i>	<i>Clark T. Eidsmoe</i>
1920-21	<i>Ivar Griffith</i>	<i>H. M. Faser</i>			

SECTION ON PRACTICAL PHARMACY

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1941-42	<i>W. A. Prout</i>	<i>Clark T. Eidsmoe</i>	1950-51	<i>Raymond E. Schmitz</i>	<i>Elmer M. Plein</i>
1942-44	<i>E. P. Guth</i>	<i>R. W. Clark</i>	1951-52	<i>Mary K. Keenan</i>	<i>Elmer M. Plein</i>
1944-46	<i>G. E. Crassen</i>	<i>A. P. Wyss</i>	1952-53	<i>Elmer M. Plein</i>	<i>S. W. Goldstein</i>
1946-47	<i>J. B. Sprowls</i>	<i>Elmer M. Plein</i>	1953-54	<i>Gordon A. Bergy</i>	<i>S. W. Goldstein</i>
1947-48	<i>Charles V. Selby</i>	<i>Elmer M. Plein</i>	1954-55	<i>H. G. DeKay</i>	<i>S. W. Goldstein</i>
1948-49	<i>John Zugich</i>	<i>Elmer M. Plein</i>	1955-56	<i>W. R. Lloyd</i>	<i>S. W. Goldstein</i>
1949-50	<i>Arthur P. Wyss</i>	<i>Elmer M. Plein</i>	1956-57	<i>L. M. Ohmolt</i>	<i>S. W. Goldstein</i>
			1957-58	<i>S. W. Goldstein</i>	<i>G. J. Sperandio</i>

SECTION ON EDUCATION AND LEGISLATION

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1889-90	<i>P. W. Bedford</i>	<i>A. B. Stevens</i>	1921-22	<i>W. H. Zeigler</i>	<i>W. F. Gidley</i>
1890-91	<i>William Simon</i>	<i>L. C. Hogon</i>	1922-23	<i>W. F. Gidley</i>	<i>C. A. Bergy</i>
1891-92	<i>A. B. Stevens</i>	<i>L. C. Hogon</i>	1923-24	<i>G. A. Bergy</i>	<i>William Mansfield</i>
1892-93	<i>R. G. Eccles</i>	<i>L. C. Hogon</i>	1924-25	<i>William Mansfield</i>	<i>J. G. Beard</i>
1893-94	<i>R. G. Eccles</i>	<i>L. C. Hogon</i>	1925-26	<i>J. G. Beard</i>	<i>H. M. Faser</i>
1894-95	<i>James M. Good</i>	<i>C. S. N. Hallberg</i>	1926-27	<i>H. M. Faser</i>	<i>M. N. Ford</i>
1895-96	<i>C. S. N. Hallberg</i>	<i>Jos. H. Beol</i>	1927-28	<i>M. N. Ford</i>	<i>A. L. I. Winne</i>
1896-97	<i>C. S. N. Hallberg</i>	<i>Jos. H. Beol</i>	1928-29	<i>A. L. I. Winne</i>	<i>Glenn L. Jenkins</i>
1897-98	<i>James H. Beal</i>	<i>H. G. Webster</i>	1929-30	<i>Glenn L. Jenkins</i>	<i>R. H. Raabe</i>
1898-99	<i>A. B. Lyons</i>	<i>C. B. Lowe</i>	1930-31	<i>B. V. Christensen</i>	<i>C. M. Anderson</i>
1899-00	<i>C. B. Lowe</i>	<i>J. A. Kach</i>	1931-32	<i>R. H. Raabe</i>	<i>C. W. Ballard</i>
1900-01	<i>C. B. Lowe</i>	<i>J. A. Koch</i>	1932-33	<i>W. H. Rivard</i>	<i>C. W. Ballard</i>
1901-02	<i>E. G. Eberle</i>	<i>J. W. T. Knax</i>	1933-34	<i>George C. Schicks</i>	<i>C. W. Ballard</i>
1902-03	<i>J. W. T. Knax</i>	<i>Horry B. Masan</i>	1934-35	<i>Oscar E. Russell</i>	<i>L. W. Rising</i>
1903-04	<i>Harry B. Masan</i>	<i>Wm. L. Cliffe</i>	1935-36	<i>C. Leonard O'Cannell</i>	<i>G. A. Moulton</i>
1904-05	<i>Harry B. Masan</i>	<i>Wm. L. Cliffe</i>	1936-37	<i>George C. Schicks</i>	<i>John F. McClaskey</i>
1905-06	<i>Oscar Oldberg</i>	<i>Jas. W. England</i>	1937-38	<i>Gea. A. Maultan</i>	<i>A. O. Mickelsen</i>
1906-07	<i>Oscar Oldberg</i>	<i>Jos. W. England</i>	1938-39	<i>J. F. McClaskey</i>	<i>Leslie M. Ohmart</i>
1907-08	<i>Jos. W. England</i>	<i>C. H. LaWall</i>	1939-40	<i>A. O. Mickelsen</i>	<i>R. T. Lakey</i>
1908-09	<i>Jas. W. England</i>	<i>C. H. LaWall</i>	1940-41	<i>Leslie M. Ohmart</i>	<i>F. J. Goadrich</i>
1909-10	<i>C. H. LaWall</i>	<i>Chas. W. Jahnsan</i>	1941-42	<i>R. T. Lakey</i>	<i>E. J. Ireland</i>
1910-11	<i>Chas. W. Jahnsan</i>	<i>W. J. Teeters</i>	1942-44	<i>E. J. Ireland</i>	<i>P. O. Clark</i>
1911-12	<i>John C. Wallace</i>	<i>W. J. Teeters</i>	1944-46	<i>W. F. Sudro</i>	<i>B. A. Bialk</i>
1912-13	<i>W. J. Teeters</i>	<i>Frank H. Freericks</i>	1946-47	<i>J. S. Lucas</i>	<i>B. A. Bialk</i>
1913-14	<i>Hugh Craig</i>	<i>Frank H. Freericks</i>	1947-48	<i>James S. Hill</i>	<i>Ralph W. Clark</i>
1914-15	<i>F. H. Freericks</i>	<i>R. A. Kuever</i>	1948-49	<i>B. A. Bialk</i>	<i>Ralph W. Clark</i>
1915-16	<i>F. H. Freericks</i>	<i>R. A. Kuever</i>	1949-50	<i>Ralph W. Clark</i>	<i>David W. O'Day</i>
1916-17	<i>R. A. Kuever</i>	<i>C. B. Jordan</i>	1950-51	<i>David W. O'Day</i>	<i>J. L. Voigt</i>
1917-18	<i>C. B. Jordan</i>	<i>W. F. Rudd</i>	1951-52	<i>C. Lee Huyek</i>	<i>Ralph Mill</i>
1918-19	<i>W. F. Rudd</i>	<i>C. A. Dye</i>	1952-53	<i>J. L. Voigt</i>	<i>R. A. Walsh</i>
1919-20	<i>C. A. Dye</i>	<i>Edward Spease</i>	1953-54	<i>R. J. Mill</i>	<i>F. L. Mercer</i>
1920-21	<i>Edward Spease</i>	<i>W. H. Ziegler</i>	1954-55	<i>R. A. Walsh</i>	<i>Hugh Ferguson</i>
			1955-56	<i>F. L. Mercer</i>	<i>Albert Picchiani</i>
			1956-57	<i>H. C. Ferguson</i>	<i>J. R. McCowan</i>
			1957-58	<i>A. L. Picchiani</i>	<i>R. K. Mulvey</i>

OFFICERS OF THE SECTIONS (Continued)

SECTION ON COMMERCIAL INTERESTS

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1887-88	<i>A H Hollister</i>	<i>J W Colcard</i>	1912-13	<i>Autumn V Pease</i>	<i>William R White</i>
1888-89	<i>A H Hollister</i>	<i>J W Colcard</i>	1913-14	<i>C G Lindvall and</i> <i>H B Mason</i>	<i>Grant W Stevens</i>
1889-90	<i>Leo Elsel</i>	<i>F B Kilmer</i>			
1890-91	<i>Henry Canning</i>	<i>W L Dewaody</i>	1914-15	<i>E H Thiesing</i>	<i>David Stolz</i>
1891-92	<i>W H Torbert</i>	<i>Arthur Bassell</i>	1915-16	<i>R S Lehman</i>	<i>J C McGee</i>
1892-93	<i>W H Torbert</i>	<i>Arthur Bassell</i>	1916-17	<i>P Henry Utech</i>	<i>Robert P Fischelis</i>
1893-94	<i>Wiley Rogers</i>	<i>Jas O Burge</i>	1917-18	<i>Robert P Fischelis</i>	<i>F W Nitarady</i>
1894-95	<i>Geo J Seabury</i>	<i>Jas O Burge</i>	1918-19	<i>E Fullerton Cook</i>	<i>H S Noel</i>
1895-96	<i>Geo J Seabury</i>	<i>Clayton W Halmes</i>	1919-20	<i>H S Noel</i>	<i>C O Lee</i>
1896-97	<i>Lewis C Happ</i>	<i>E D Avignon</i>	1920-21	<i>Adam Wirth</i>	<i>Charles W Holton</i>
1897-98	<i>Joseph Jacobs</i>	<i>Jas H Babbitt</i>	1921-22	<i>Charles W Holton</i>	<i>B H Eschold</i>
1898-99	<i>Joseph Jacobs</i>	<i>Jas H Babbitt</i>	1922-23	<i>Walter M Chase</i>	<i>Henry B Smith</i>
1899-00	<i>James M Goad</i>	<i>Charles A Rapelye</i>	1923-24	<i>Henry B Smith</i>	<i>W Bruce Philp</i>
1900-01	<i>Charles A Rapelye</i>	<i>F W Meissner</i>	1924-25	<i>W Bruce Philp</i>	<i>George Judisch</i>
1901-02	<i>F W Meissner</i>	<i>E G Eberle</i>	1925-26	<i>Ambrose Hunsberger</i>	<i>B M Keene</i>
1902-03	<i>T V Woaten</i>	<i>Wm C Anderson</i>	1926-27	<i>B M Keene</i>	<i>C L O'Connell</i>
1903-04	<i>Wm L Dewaody</i>	<i>Robert C Reilly</i>	1927-28	<i>C L O'Connell</i>	<i>R B Rothrock</i>
1904-05	<i>Charles R Sherman</i>	<i>Robert C Reilly</i>	1928-29	<i>R B Rothrock</i>	<i>J G Noh</i>
1905-06	<i>Henry P Hynson</i>	<i>H D Kniseley</i>	1929-30	<i>Denny Brann</i>	<i>Rowland Jones</i>
1906-07	<i>H D Kniseley</i>	<i>Charles H Atery</i>	1930-31	<i>Jos G Noh</i>	<i>Leon Monell</i>
1907-08	<i>Jacob Diner</i>	<i>George O Young</i>	1931-32	<i>Rowland Jones</i>	<i>John A J Funk</i>
1908-09	<i>Harry B Mason</i>	<i>Erich H Ladish</i>	1932-33	<i>L M Monell</i>	<i>Henry Brown</i>
1909-10	<i>W M Bowman</i>	<i>G H P Lichthardt</i>	1933-34	<i>John A J Funk</i>	<i>Robert W Rodman</i>
1910-11	<i>Franklin M Apple</i>	<i>Benj E Prichard</i>	1934-35	<i>Henry Brown</i>	<i>R T Lakey</i>
1911-12	<i>Ernest Berger</i>	<i>D W Ramsaur</i>	1935-36	<i>R W Rodman</i>	<i>H F Hein</i>
			1936-37	<i>R T Lakey</i>	<i>J H Goodness</i>

SECTION ON PHARMACEUTICAL ECONOMICS

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1937-38	<i>Henry F Hein</i>	<i>Joseph H Goodness</i>	1948-49	<i>W L Cahiff</i>	<i>E J Ireland</i>
1938-39	<i>Paul C Olsen</i>	<i>Joseph H Goodness</i>	1949-50	<i>Edward Ireland</i>	<i>J A MacCartney</i>
1939-40	<i>Joseph H Goodness</i>	<i>C M Brown</i>			
1940-41	<i>C M Brown</i>	<i>Ira Rothrock</i>	1950-51	<i>John A MacCartney</i>	<i>Francis J O'Brien</i>
1941-42	<i>B Olive Cole</i>	<i>H W Heine</i>	1951-52	<i>Francis J O'Brien</i>	<i>Alvah G Hall</i>
1942-44	<i>B R Mull</i>	<i>Stephen Wilson</i>	1952-53	<i>Alvah G Hall</i>	<i>Irving Rubin</i>
1944-46	<i>B R Mull</i>	<i>Stephen Wilson</i>	1953-54	<i>Irving Rubin</i>	<i>J W Lansdowne</i>
1946-47	<i>Stephen Wilson</i>	<i>G F Archamhault</i>	1954-55	<i>J W Lansdowne</i>	<i>Noel M Ferguson</i>
1947-48	<i>Stephen Wilson</i>	<i>E J Ireland</i>	1955-56	<i>Noel M Ferguson</i>	<i>Chas C Rabe</i>
			1956-57	<i>G L Scharringhausen Jr</i>	<i>B A Smith</i>
			1957-58	<i>H W Pratt</i>	<i>B A Smith</i>

SECTION ON HISTORICAL PHARMACY

Year	Chairmen	Secretaries	Year	Chairmen	Secretaries
1904-05	<i>Albert E Eberle</i>	<i>Caswell A Mayo</i>	1930-31	<i>J T Lloyd</i>	<i>L E Warren</i>
1905-06	<i>John F Hancock</i>	<i>C S N Hallberg</i>	1931-32	<i>J T Lloyd</i>	<i>Louis Gershenfeld</i>
1906-07	<i>Ewen McIntyre</i>	<i>Eugene G Eberle</i>	1932-33	<i>Louis Gershenfeld</i>	<i>C O Lee</i>
1907-08	<i>E V Howell</i>	<i>Eugene G Eberle</i>	1933-34	<i>Louis Gershenfeld</i>	<i>C O Lee</i>
1908-09	<i>Jahn B Bana</i>	<i>Eugene G Eberle</i>	1934-35	<i>C O Lee</i>	<i>H W Youngken</i>
1909-10	<i>Eugene G Eberle</i>	<i>Jahn A Dunn</i>	1935-36	<i>H W Youngken</i>	<i>L E Harris</i>
1910-11	<i>Jas L Lemberger</i>	<i>O Raubenheimer</i>	1936-37	<i>Lloyd E Harris</i>	<i>E J Ireland</i>
1911-12	<i>O Raubenheimer</i>	<i>Caswell A Mayo</i>	1937-38	<i>E J Ireland</i>	<i>W T Bradley</i>
1912-13	<i>John G Godding</i>	<i>F T Gardan</i>	1938-39	<i>Willis T Bradley</i>	<i>J Hampton Hoch</i>
1913-14	<i>W C Alpers</i>	<i>F T Gordon</i>	1939-40	<i>J Hampton Hoch</i>	<i>L F Jones</i>
1914-15	<i>F T Gordon</i>	<i>A H Clark</i>	1940-41	<i>Ivor Griffith</i>	<i>F D Stoll</i>
1915-16	<i>Charles Halshauer</i>	<i>G G Marshall</i>	1941-42	<i>L F Jones</i>	<i>R D Bienfang</i>
1916-17	<i>W L DuBos</i>	<i>L E Sayre</i>	1942-43	<i>F D Stoll</i>	<i>K L Kaufman</i>
1917-18	<i>L E Sayre</i>	<i>Huga Kantrowitz</i>	1943-44	<i>F D Stoll</i>	<i>K L Kaufman</i>
1918-19	<i>Huga Kantrowitz</i>	<i>W O Richtmann</i>	1944-46	<i>F D Stoll</i>	<i>K L Kaufman</i>
1919-20	<i>W O Richtmann</i>	<i>Curt P Wimmer</i>	1946-47	<i>K L Kaufman</i>	<i>K Redman</i>
1920-21	<i>Curt P Wimmer</i>	<i>A W Lintan</i>	1947-48	<i>C M Brown</i>	<i>R T Lakey</i>
1921-22	<i>Curt P Wimmer</i>	<i>E G Eberle</i>	1948-49	<i>Kenneth Redman</i>	<i>H George Wolfe</i>
1922-23	<i>Clyde L Eddy</i>	<i>Rabi S Lehman</i>	1949-50	<i>Roland T Lakey</i>	<i>Glenn Sonnedecker</i>
1923-24	<i>Clyde L Eddy</i>	<i>Rabi S Lehman</i>	1950-51	<i>H George Wolfe</i>	<i>Edward S Brady</i>
1924-25	<i>Robt S Lehman</i>	<i>L K Darbaker</i>	1951-52	<i>Glenn Sonnedecker</i>	<i>George B Griffenhagen</i>
1925-26	<i>L K Darbaker</i>	<i>W F Sudro</i>	1952-53	<i>E S Brady</i>	<i>E J Rowe</i>
1926-27	<i>W F Sudra</i>	<i>E J Kennedy</i>	1953-54	<i>George B Griffenhagen</i>	<i>G E Osborne</i>
1927-28	<i>W P Parterfield</i>	<i>Ambrose Mueller</i>	1954-55	<i>E J Rowe</i>	<i>George B Griffenhagen</i>
1928-29	<i>L F Kehler</i>	<i>Geo D Beal</i>	1955-56	<i>G E Osborne</i>	<i>George B Griffenhagen</i>
1929-30	<i>Geo D Beal</i>	<i>J T Lloyd</i>	1956-57	<i>E R Bonow</i>	<i>George B Griffenhagen</i>
			1957-58	<i>Alex Berman</i>	<i>George B Griffenhagen</i>

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American Association for the Advancement of Science	Dael Wolfe Executive Officer, 1515 Massachusetts Ave., N W, Washington 5 D C	American Medical Association	George F Lull, 535 N Dearborn St., Chicago 10, Ill
American Chemical Society	Alden H Emery, 1155 Sixteenth St., N W, Washington 6, D C	American Nurses Association	Ella Best, Ex Sec., 2 Park Ave New York 16 N Y
American Dental Association	Harold Hillenbrand, 222 E Superior St., Chicago 11 Ill	American Public Health Association	Berwyn F Mattison 1790 Broadway New York 19
American Hospital Association	Edwin L Crosby, Ex Dir., 18 E Division St., Chicago 10, Ill	American Social Hygiene Association	Conrad Van Hyming, 1790 Broadway, New York, N Y
		American Veterinary Medical Association	J G Hardenbergh 600 S Michigan Ave., Chicago 5
		National Health Council	Philip E Ryan Ex Dir., 1790 Broadway, New York 19

SECTIONAL, STATE, AND LOCAL PUBLICATIONS

NAME	EDITOR OR MANAGER	NAME	EDITOR OR MANAGER
Allegheny County Pharmacist	Wm H Whitman 2200 5th Ave, Pittsburgh 19, Pa	Missouri Pharmacist	John Hoshor, 601 Central Trust Bldg, Jefferson City, Mo
Apothecary	John L Heaton 376 Boylston St Boston 16 Mass	National Capital Pharmacist	Harold C Kinner, 145 Kennedy St, N W, Suite 5 United Bldg, Washington D C
Arizona Pharmacist	A J Duncan 1028 E McDowell St, Phoenix Ariz	Nebraska Mortar and Pestle	Cora Mae Briggs, 410 Fed Sec Bldg, Lincoln 8, Nebr
Arkansas Pharmacist	Wm G Smith, 607 Wallace Bldg, Little Rock, Ark	New Jersey Journal of Pharmacy	A Charles Corotis, 118 W State St, Trenton 8 N J
C R D A News	John M Myers 32 W Randolph St Chicago Ill	New York State Pharmacist	David Beiles, 117 E 69th St, New York 21, N Y
California Pharmacy	James W Gentry 701 So St Andrews Place Los Angeles 5 Calif	Northern California Drug News	Geo F Bohlken, 525 Market St, San Francisco 5 Calif
Carolina Journal of Pharmacy	W J Smith, Box 151 Chapel Hill, N C	Northwestern Druggist	E M Dockstader, 2642 University Ave, St Paul Minn
Central Pharmaceutical Journal	Joseph J Shune, 221 N La Salle St Chicago 1, Ill	Ohio Pharmacist	Jas D Cope, 33 N High St Columbus 15, Ohio
Cincinnati Academy of Pharmacy News	J F Donahue 7404 Juler Ave Cincinnati 43 Ohio	O V D A Review	Harold C Frelking, 1014 Race St, Cincinnati 2 Ohio
Cleveland Academy of Pharmacy Journal	Larry A Shatten 1935 Euclid Ave Cleveland 15 Ohio	Oklahoma Pharmacist	Elbert R Weaver, 620 Main St, Stillwater, Okla
Connecticut Pharmacist	Raymond E Mercier 196 Greenway St Hamden 15, Conn	Oregon Pharmacist	H A Speckman, 709 Jackson Tower, Portland 5, Oreg
Delaware Pharmacist	Harry C Zeisig 407 Delaware Ave, Wilmington, Del	P A R D Bulletin	Hymen C Bogash, 2017 Spring Garden St, Philadelphia 30 Pa
Delaware County (Penna) Pharmacist	A M Lauter, 1582 Chestner Ave, Linwood Pa	Pacific Drug Review	W C Felter, 504 Woodlark Bldg, Portland 5, Oreg
Drug Progress	Thomas J. Vratny, 7 South Dearborn St, Chicago Ill	Pennsylvania Pharmacist	Sam Price, 208 W Third St Williamsport, Pa
El Boticario	E L Cataline, Editor, 122 Harvard Dr S E Albuquerque, N M	Rocky Mountain Druggist	Verne N Seeley, 1441 Welton St, Denver 2, Colo
Florida Pharmaceutical Journal	R Q Richards Fort Myers, Fla	St Louis Retail Druggists News	Herm Winkelmann, 5101 Hampton St Louis 9, Mo
Indiana Pharmacist	Henry W Heine 54 Monument Circle Indianapolis 4, Ind	South Dakota Journal of Medicine and Pharmacy	R G Mayer, Aherdeen, S Dak
Iowa Pharmacist	Dal Bruner, 540 Des Moines Bldg, Des Moines 9, Iowa	South Jersey Pharmacist	A Charles Corotis 1 Clinton St, Newark, N J
K P A News	Clara B Miller, 824 Kansas Ave, Topeka, Kans	Southeastern Drug Journal	James H Bishop, Jr, 423 Grand Theatre Bldg, Atlanta 3 Ga
Kentucky Pharmacist	E M Josey, 213 St Clair St, Frankfort, Ky	Southern Pharmaceutical Journal	Walter Cousins Jr 518 Interurban Bldg, Dallas 1 Tex
Louisiana Pharmacist	Richard G Drown, Jr, 219 Carondelet Bldg, New Orleans 12, La	Texas Druggist	M Roesch, 265 Texas St Fort Worth, Tex
Maryland Pharmacist	Joseph Cohen, 650 W Lombard St, Baltimore 1, Md	Utah Pharmaceutical Association Bulletin	H Ward McCarty Suite 221, 1086 E 21st, South, Salt Lake City 6, Utah
Michigan Journal	R T Lakey, 709 Francis Palms Bldg, Detroit 1, Mich	Virginia Pharmacist	James O Hubbard, Jr, 1105 East Clay St, Richmond, Va
Mid Atlantic Apothecary	James S Talbott, 5th & Jersey Ave, Gloucester, N J	West Coast Druggist	Bert Butterworth, 1606 N Highland Ave, Hollywood 28, Calif
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Minnesota Pharmacist	Henry M Moen 2388 University Ave, St Paul, Minn	Wisconsin Druggist	Jennings Murphy, 161 W Wisconsin Ave, Milwaukee, Wis

CONFERENCES AND OTHER ORGANIZATIONS

NAME	SECRETARY OR DIRECTOR	NAME	SECRETARY OR DIRECTOR
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American Foundation for Pharmaceutical Education	W Paul Briggs, 1507 M St, N W, Washington 5, D C	National Conference of State Pharmaceutical Associations Secretaries	William J Dixon, Box 119 Oak Hill, W Va
Commission on Professional Manpower for Pharmacy	Robert P Fischels, Chairman, 2215 Constitution Ave, N W, Washington 7, D C	National Drug Trade Conference	R C Schlotterer, 2 Lexington Ave, New York 10, N Y
Federation Internationale Pharmaceutique	Secretary General Dr J W Burza, 11 Alexanderstraat, The Hague (Netherlands)	Pan American Federation	Dr Leonard Piccoli, 745 Fifth Avenue, New York 22, N Y
Health Information Foundation	George Bughee President, 420 Lexington Ave, New York 17, N Y	The Plant Science Seminar	Dr Frank L Mercer, St Louis College of Pharmacy, St Louis 10, Mo
Health News Institute	Chet Shaw, Director, 60 East 42nd St, New York 17, N Y	United States Pharmacopoeial Convention	Adley B Nichols 46 Park Ave, New York 16, N Y

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ACTIVE MEMBERS

The list corrected to December 31, 1957

Associate (Student) Members are not listed. The names of Life Members are in capitals. The number in parentheses after each name indicates the year continuous membership began. This list includes active members in good standing as of December 31, 1957, and also those members who were not listed in any previous printed list, but who may not have continued to be in good standing up to December 31, 1957. Such arrangements have been made to assure continuation of availability of a published list of all members in one of the ASSOCIATION publications since its origin.

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add 9 ml. of absolute ethyl alcohol and 0.3 ml. of triethylamine, and make to volume with chloroform.

(2) **Chromatographic Column.**—The column is made of a 25 × 200 ml. test tube to the bottom of which is attached an approximately 5 cm. length of 6–8 mm. tubing. The tamping rod consists of a disk of stainless steel, aluminum, or glass, with a diameter 1 mm. less than that of the column, attached to a rod 12 to 18 inches long. Pack fine glass wool in the base of the column to act as a support.

To 2.0 Gm. of Celite 545 (Johns-Manville Corp.) in a mortar add 2.0 ml. of approximately 1 *N* NaHCO₃ and incorporate thoroughly by kneading with a flexible spatula blade. Transfer to column and tamp, using gentle pressure, to compress the material to a uniform mass. This constitutes column *A*. In a like manner, mix 1.0 Gm. of Celite with 0.5 ml. of concentrated NH₄OH, and pack in a second column. Above this place a mixture of 2.0 Gm. of Celite with 2.0 ml. of 4 *N* H₂SO₄, followed by 2.0 Gm. of Celite mixed with 2.0 ml. of 1 *N* tartaric acid. This constitutes column *B*, used for APC-organic base combinations. For APC-barbiturate combinations, column *C* is prepared with a lower segment composed of 2.0 Gm. of Celite with 2.0 ml. of 4 *N* H₂SO₄ and the upper segment containing 2.0 Gm. of Celite with 2.0 ml. of 1 *N* K₃PO₄.

A. Separation of APC-Organic Base Combinations.—(Use water-washed solvents in the ensuing procedure). Mount columns *A* and *B* in such a manner that the effluent from column *A* flows directly into column *B*. Wash with 15–20 ml. of ether, discarding the washings.

Without filtering the sample preparation, withdraw a 5-ml. or 10-ml. aliquot (depending upon the content of the components) and dilute with 4 volumes of ether. Allow solution to pass through columns. After the last portion has passed onto the adsorbent, wash with five 5-ml. portions of ether, allowing each to pass onto the adsorbent prior to the addition of the ensuing one. Evaporate the combined eluate to dryness on a steam bath in a current of air. This fraction contains the acetophenetidin. Dissolve the residue in 5 ml. of chloroform and make to volume with iso-octane in a 50 ml. volumetric flask. Make any dilution necessary to adjust to a concentration suitable for spectrophotometric measurement with a 1:10 mixture of chloroform and iso-octane.

Immediately after passage of the last portion of ether through the columns, replace the receiver with a 50-ml. volumetric flask. Pass 48 ml. of chloroform through columns and dilute to volume. This fraction contains caffeine, which is determined spectrophotometrically after proper dilution with chloroform.

Separate columns *A* and *B*. Place a 100-ml. volumetric flask as receiver for column *A* and pass a solution of 0.5 ml. of glacial acetic acid in 5 ml. of chloroform through the column followed by 92 ml. of a 1% solution of acetic acid in chloroform. Adjust to volume and make proper dilutions with a 1% acetic acid-chloroform solution for the determination of acetylsalicylic acid.

Add to column *B* (except in the case of phenindamine) a solution of 2 ml. of redistilled triethylamine in 5 ml. of chloroform followed by 100 ml. of a 1% solution of triethylamine in chloroform.

Evaporate the eluate to dryness on a steam bath in a current of air, heating two to five minutes after evaporation of solvent to insure complete removal of the triethylamine. For samples containing phenindamine, elute with chloroform saturated with ammonia, prepared by shaking 100 ml. of chloroform with 25 ml. of concentrated ammonium hydroxide. When evaporating this eluate, remove from heat immediately upon the removal of the last trace of solvent.

Dissolve the residue in 10 ml. of water containing 5 to 10 drops of hydrochloric acid, and adjust to a suitable volume with water for the determination of the organic base.

B. For APC-barbiturate combinations.—Mount columns *A* and *C* as above and proceed as described above for the elution of acetophenetidin, caffeine, and acetylsalicylic acid. For the elution of the barbiturate, add to column *C*, 0.5 ml. of glacial acetic acid in 5 ml. of chloroform, followed by 95 ml. of a 1% solution of acetic acid in chloroform. Evaporate to dryness. Dissolve the residue in 10 ml. of water containing 10 drops of ammonium hydroxide, and adjust to a suitable volume with water.

In the spectrophotometric determination of all of the ingredients separated, read the absorbances of the solutions prepared as above and compare the values obtained with those simultaneously determined on corresponding standard solutions. Chloroform containing 1% acetic acid is used for solutions of salicylic and acetylsalicylic acids and the latter standard must be prepared daily.

As a guide in determining the proper dilutions, the values in Table I may be used.

RESULTS

Mixtures of acetophenetidin, caffeine, acetylsalicylic acid, and the associated compounds, in ratios approximating those found in the commercial

TABLE I

Compound	Wave-length μ	Concentration mcg./ml.	Absorbance (Approximate)
Acetophenetidin	285	50	0.505
Caffeine	276	10	0.485
Acetylsalicylic Acid	280	100	0.790
	310	100	0.010
Salicylic Acid ^a	280	25	0.113
	310	25	0.573
Pyrimidine Maleate	315	10	0.205
Chlorophenylpyridamine Maleate	265	10	0.212
Phenindamine Tartrate	260	10	0.219
Methapyrilene Hydrochloride	314	10	0.274
Doxylamine Succinate	261	10	0.215
Thonzylamine Hydrochloride	314	10	0.102
Codeine Sulfate	284	100	0.444
Codeine Phosphate	284	100	0.395
Phenobarbital	240	10	0.445
Cyclopentenylalyl Barbituric Acid	241	10	0.366

^a Calculated as acetylsalicylic acid. For calculation of total acetylsalicylic acid in mixture with partially hydrolyzed material see (1).

TABLE II

Associated Compound			Acetophenetidin		Caffeine		Acetylsalicylic Acid	
	Added Mg	Found Mg	Added Mg	Found Mg	Added Mg	Found Mg	Added Mg	Found Mg
Pyrilamine Maleate	1 00	0 99	10 40	10 49	2 00	1 99	17 00	16 52
	1 00	1 00	11 90	11 89	2 00	2 00	17 30	17 03
Phenindamine Tartrate	1 00	1 00	29 50	29 56	2 00	2 00	38 00	37 59
	1 00	0 98	29 80	30 05	2 00	2 00	29 40	29 11
Thonzylamine Hydrochloride ^a	1 00	1 02	5 00	5 09	2 00	1 99	10 40	10 22
	1 00	1 02	5 00	5 08	2 00	1 97	10 90	10 63
Doxylamine Succinate	1 00	1 01	24 80	24 50	5 00	4 96	40 50	40 51
	1 00	1 02	25 50	25 29	5 00	5 01	49 80	49 87
Methapyrilene Hydrochloride	1 00	1 00	5 00	5 03	2 00	1 98	10 20	10 09
	1 00	1 00	5 00	4 94	2 00	1 98	12 06	11 74
Chlorophenpyridamine Maleate	0 50	0 51	40 00	40 10	10 00	9 92	62 50	63 70
	0 50	0 50	42 00	42 25	10 00	9 94	60 40	60 00
Codeine Sulfate	2 50	2 55	19 00	18 87	3 00	3 01	31 70	31 08
	2 50	2 49	20 90	21 03	3 00	3 01	24 00	23 29
Phenobarbital	2 50	2 51	25 10	24 86	5 00	4 90	40 30	40 51
	2 50	2 53	25 60	25 05	5 00	4 92	34 60	34 56
Cyclopentenylallyl Barbituric Acid ^b	2 28	2 23	26 00	25 78	5 00	4 80	34 10	34 11
	2 28	2 24	26 30	26 32	5 00	4 94	36 00	36 01

^a Mixture contains 1 mg ascorbic acid^b Sodium salt added

TABLE III

Associated Compound			Acetophenetidin		Caffeine		Acetylsalicylic Acid	
	Declared Mg/Tab	Found Mg/Tab	Declared Gr/Tab	Found Gr/Tab	Declared Gr/Tab	Found Gr/Tab	Declared Gr/Tab	Found Gr/Tab
Pyrilamine Maleate	12 5	12 03	2 50	2 50	0 50	0 50	3 50	3 47
		12 13		2 45		0 49		3 44
Phenindamine Tartrate	10 0	9 87	160 0 mg	161 2 mg	15 0 mg	14 84 mg	160 0 mg	160 2 mg
		9 86		159 7		14 89		161 5
Thonzylamine Hydrochloride ^a	25 0	24 65	1 50	1 46	0 50	0 50	3 50	3 41
		24 56		1 46		0 50		3 41
Doxylamine Succinate	6 0	5 75	2 50	2 31	0 50	0 43	3 50	3 34
		5 69		2 32		0 44		3 36
Methapyrilene Hydrochloride	25 0	24 09	2 50	2 44	0 50	0 47	3 50	3 47
		24 32		2 43		0 48		3 41
Chlorophenpyridamine Maleate	2 0	1 85	2 50	2 53	0 50	0 50	3 50	3 56
		1 82		2 52		0 50		3 55
Codeine Sulfate ^b	0 50 gr	0 49 gr.	2 50	2 48	0 50	0 48	3 50	3 35
		0 48		2 51		0 48		3 38
Codeine Phosphate	0 50 gr	0 51 gr.	2 50	2 51	0 50	0 51	3 50	3 49
		0 50		2 51		0 50		3 45
Phenobarbital	0 25 gr	0 25 gr	2 50	2 49	0 00	0 00	3 50	3 47
		0 25		2 45		0 00		3 50
Cyclopentenylallyl Barbituric Acid	0 75 gr	0 77 gr.	2 50	2 51	0 50	0 49	3 50	3 43
		0 78		2 50		0 49		3 47

^a Contain 20 mg ascorbic acid per tablet^b Contain flavoring additives

products, were assayed according to the above procedure. Results are reported in Table II. The assays of commercial tablets are reported in Table III. The duplicate assays represent separate samples, not duplicate aliquots withdrawn from the same sample preparation.

No difficulty was introduced by any of the excipients. In one instance in which the tablet contained a very large proportion of chloroform-insoluble excipient, aliquots were withdrawn both immediately after shaking the sample preparation and after allowing the insoluble matter to precipitate, no difference was found in the results.

SUMMARY

Tablets containing acetylsalicylic acid, acetophenetidin, and caffeine combined with codeine, barbiturates, or antihistaminic drugs may be separated into their component compounds by a modified partition chromatographic procedure. Acetophenetidin, which is neutral, passes through the chromatographic column in chloroform-ether solution. Caffeine, a feebly basic alkaloid, is trapped by sulfuric acid, from which it is eluted

with chloroform Acetylsalicylic acid is trapped by sodium bicarbonate; the barbiturates, which are weakly acidic, pass through the sodium bicarbonate but are trapped by tripotassium phosphate. Both are eluted with a solution of acetic acid in chloroform Codeine and the antihistamines are trapped by tartaric acid and sulfuric acid from which they are eluted with a solution of triethylamine in chloroform. The procedure is

rapid, an entire analysis can be completed in approximately one and one-half hours.

The concentrations of the components are measured spectrophotometrically.

REFERENCES

- (1) Levine, J, *THIS JOURNAL*, 46, 687(1957)
- (2) Banes, D, *J Assoc Official Agr Chem*, 34, 80, 566 (1951)

Biosynthesis of C¹⁴-Reserpine by *Rauwolfia* Plants*

By EDWIN A. PEETS,† ARTHUR R. SCHULERT,† JOHN SKOK,‡ and WILLIAM CHORNEY‡

Rauwolfia plants were grown in an atmosphere containing C¹⁴O₂ for the purpose of biosynthesizing randomly labeled C¹⁴-reserpine for use in further metabolic studies. Preliminary experiments indicated that the activity appearing in reserpine, expressed as percentage of the total activity fixed by plant, was lowest in plants started from cuttings, higher in old seedling plants and highest in young seedling plants. A total of 64.5 millicuries C¹⁴ was incorporated into *Rauwolfia* plants to produce approximately 225 mg. of reserpine containing 98 microcuries of C¹⁴ with a specific activity of 670 microcuries per Gm. of carbon.

RESERPINE, the crystalline indole alkaloid extracted from the roots of plants of the genus *Rauwolfia* (1) has tranquilizing effects in psychiatric disorders as well as hypotensive action. For this reason a great deal of interest has been directed to its metabolism and site of action. The use of a C¹⁴-labeled form of the drug would greatly facilitate attempts to obtain such information. Investigations have been made using side-chain-labeled reserpine (2, 3), but a randomly labeled form would have the advantage of permitting study of the whole molecule, including the

ring moiety. Such randomly labeled reserpine was prepared biosynthetically by supplying *Rauwolfia* plants with C¹⁴O₂.

PROCEDURES

Materials and Facilities.—Four species of *Rauwolfia* (*serpentina*, *hirsuta*, *verticillata*, and *vomitoria*) were obtained from the Plant Introduction Section of the United States Department of Agriculture.¹ The plants were maintained in the greenhouse and grown in soil. They were prepared for use in biosynthesis by washing the soil from the roots, transplanting the plants to sand, and supplying them with nutrient solutions. The biosynthesis of C¹⁴-reserpine was carried out by growing the plants in a specially constructed chamber (4) designed for this purpose. The chamber, located in a greenhouse, consists of a steel frame of approximate dimensions of 5 x 7½ x 6 ft, containing glass panes embedded in a CO₂-impervious mastic. A removable panel permits entry and is sealed when in operation. The plants were grown in gravel contained in a stainless steel bed. Nutrient solutions supplied from a stainless steel storage tank by sub-irrigation contained:

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‡ Lamont Geological Observatory.

§ Argonne National Laboratory.

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$\text{Ca}(\text{NO}_3)_2$, 0.0045 M; KH_2PO_4 , 0.0023 M; MgSO_4 , 0.0023 M; B, 0.50 p.p.m.; Mn, 0.25 p.p.m.; Zn, 0.05 p.p.m.; Cu, 0.02 p.p.m.; Mo, 0.05 p.p.m.; and Fe, 0.50 p.p.m. added as Sequestrene NaFe. The temperature in the chamber was maintained at $85 \pm 1^\circ \text{F}$. by a constant flow of temperature-regulated water over the outside surfaces. The relative humidity of the chamber atmosphere was maintained at $50 \pm 5\%$. Air samples from the chamber were pumped through monitoring devices; total CO_2 was measured by an infrared gas analyzer and C^{14} activity of the air phase was measured with a vibrating diaphragm-type ionization chamber. The desired specific activity of the air phase CO_2 was maintained by periodic generation of CO_2 from a calculated ratio of $\text{BaC}^{14}\text{O}_3/\text{BaC}^{12}\text{O}_3$. The CO_2 concentration within the chamber was held near that normally present in the atmosphere, namely within the range of 0.01 to 0.06%.

Harvest, Assay, and Isolation Procedures.—At the termination of a designated culture period in a C^{14}O_2 atmosphere, the plants were removed from the chamber and the roots were carefully recovered from the gravel substrate. Plant parts were separated, dried at 70°C . in a forced air oven, and ground in a Wiley mill to pass a 30-mesh screen. Samples were counted at infinite thickness with an end-window counter and their radioactivity was determined by comparing the count rate with that of a standard plant sample. The standard consisted of C^{14} -labeled ground plant material, an aliquot of which was assayed by combustion and gas counting.

One to three-Gm. ground root samples were refluxed with 20 ml. of methanol for one hour, filtered and washed twice with hot methanol. The filtrate was partially evaporated *in vacuo* to reduce the volume and made up to 5 ml. Chromatographic examination of this extract using the methods of Banes, *et al.* (5, 6), and Carol, *et al.* (7), established the presence of five fluorescent alkaloids in addition to reserpine, namely deserpidine, reseinnamine, ajmalicine, ajmaline, and serpentine. Reserpine was separated by means of liquid-liquid partition chromatography on Celite columns (6) and assayed by ultraviolet absorption (8). Radioactivity of reserpine was determined by plating the material on a planchet, counting an infinitely thin layer in a windowless gas flow counter, and comparing its count rate with that of a similarly prepared sample of known activity.

The isolation of the labeled material was accomplished by means of a solvent extraction procedure of Korzun, *et al.* (9), rather than by chromatographic methods.

RESULTS

Preliminary Trials.—Preliminary experiments were carried out for the purpose of determining whether there were any species or plant-type differences in capacity for carbon fixation or in efficiency of incorporation of C^{14} into reserpine. Moderately mature and woody seedlings of *R. serpentina* and *R. hirsuta*, young seedlings of *R. verticillata* and young rooted cuttings of *R. verticillata* were selected. They were grown for thirteen days in an atmosphere maintained at an average specific activity of 524 microcuries per Gm. carbon in the air phase. At the end of this period the plants were harvested,

and stem, leaf and root fractions were prepared for assay in the manner described. Determinations of reserpine content and activity were also made.

The reserpine content of the roots was similar in all plants and ranged from 0.15 to 0.19%. However, the C^{14} appearing in reserpine, expressed as percentage of the total C^{14} fixed by the plant, varied: smallest amounts appeared in reserpine from roots of plants started from cuttings, higher amounts appeared in old seedlings and considerably higher amounts in young seedling plants. This may be related to the relative rate of root tissue formation and leaf respiration rates by the different types of plants. Young seedling plants are perhaps more vigorous and produce new root tissue at a higher rate than do either older seedlings or cuttings. On the basis of this information young seedling plants were used in the biosynthesis operation.

Biosynthesis of C^{14} Reserpine.—Twelve young seedlings of *R. hirsuta* and two of *R. vomitoria* were transplanted into the chamber and were permitted to become established for a one week period prior to incorporation of C^{14}O_2 . They were grown for a total of forty days in a C^{14}O_2 atmosphere with a specific activity of 1.45 millicuries per Gm. carbon. A total of 64.5 millicuries of C^{14} was fixed by the plants during this time. After a culture period of about thirty-three days under these conditions the *R. vomitoria* plants showed some radiation damage as evidenced by leaf mottling.

Of the 64.5 millicuries of C^{14} fixed by the plants, 46.0 millicuries were taken up by the 12 *R. hirsuta* plants and 18.5 millicuries by the two *R. vomitoria* plants. Of the total amount in the *R. hirsuta* plants, 49.0% appeared in the leaves, 27.7% in the stems and 26.3% in the roots. In the *R. vomitoria* plants, amounts in the comparable fractions were 33.1, 19.4 and 47.1% respectively.

A total of 75 Gm. of dry root material was obtained from the entire crop. Assays of samples of the pooled root material indicated an average reserpine content of 0.3%. The specific activity of reserpine from this pool is 670 microcuries per Gm. of carbon, giving a total available amount of 225 mg. reserpine with a total activity of 98 microcuries of C^{14} .

The reserpine was not all isolated at the same time. Isolations will be carried out as the material is needed for metabolism studies, since the material is apparently less subject to decomposition while in its natural form in the root. The labeled alkaloids are presently being used in animal studies by the first two authors.

REFERENCES

- (1) Woodson, R. E., Youngken, H. W., Schlittler, E., and Schneider, J. A., "Rauwolfia: Botany, Pharmacognosy, Chemistry and Pharmacology," Little, Brown and Company, Boston, 1957.
- (2) Numeroff, P., Gordon, M., and Kelly, J. M., *J. Pharmacol. Exptl. Therap.*, 115, 427(1955).
- (3) Sheppard, J., and Tsien, W. H., *Proc. Soc. Exptl. Biol. Med.*, 90, 437(1955).
- (4) Seully, N. J., Chorney, W., Kostal, G., Watanabe, R., Skok, J., and Glattfeld, J. W., in "International Conference on Peaceful Uses of Atomic Energy, Geneva," Vol. 12, United Nations, New York, 1955, pp. 377-385.
- (5) Banes, D., *THIS JOURNAL*, 44, 408(1955).
- (6) Banes, D., Carol, J., and Wolff, J., *ibid.*, 44, 640(1955).
- (7) Carol, J., Banes, D., Wolff, J., and Fallsheer, H. O., *ibid.*, 45, 200(1955).
- (8) Sakal, E. H., and Merrill, E. J., *ibid.*, 43, 709(1954).
- (9) Korzun, R. P., St. Andre, A. F., and Ulshaefer, P. R., *ibid.*, 46, 720(1957).

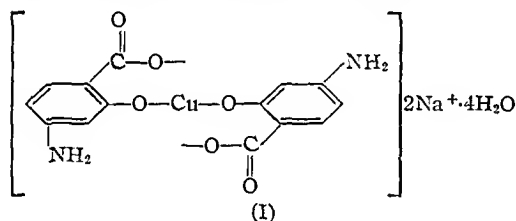
Metal Chelates and Antitubercular Activity III.*

p-Aminosalicylic Acid: Chelate *vs.* Complex

By WILLIAM O. FOYE and RONALD N. DUVALL†

Cupric and ferrous chelates of *p*-aminosalicylic acid have been isolated and characterized, and their physical and antitubercular properties compared with those of a previously isolated cupric complex. The cupric chelate showed much higher *in vivo* antitubercular activity in mice than the cupric complex, and was found to be about thirty times more fat-soluble than the cupric complex. The ferrous chelate was not appreciably active against experimental tuberculosis. The effect of these findings in explaining a possible mechanism of the antitubercular activity of *p*-aminosalicylic acid is discussed.

A COPPER COMPLEX of *p*-aminosalicylic acid (PAS) was isolated by Erlenmeyer, *et al.* (1), and was found to show equivalent or better tuberculostatic activity than PAS itself in two different culture media. The activity of both the complex and PAS was increased tenfold in the presence of excess cupric ion, however, which provides very good indication that PAS exerts its tuberculostatic action in the form of the cupric complex. The complex was believed to be cyclic, or a chelate, although later evidence (2) showed it to be an open phenolic or amine complex (I) rather than a cyclic chelate. At the same time, antitubercular tests in mice showed the complex to be less effective than PAS.



During the preparation of the copper complex, a green-black product is first visible and then changes gradually to the light green complex in alkaline media. The dark green product was isolated and postulated by Erlenmeyer as a salt, but the evidence presented here indicates it to be a copper chelate. A similar preparation using ferrous ion also produced a compound differing from the previously-prepared ferrous complex (2) and which chemical and biological evidence also indicates to be a chelate structure.

Antitubercular tests in mice, carried out at the Lilly Research Laboratories, showed a striking difference between the PAS complexes and PAS chelates. The copper chelate, when fed to tubercular mice as previously described for the complexes (2), was considerably more active than the copper complex and is superior in activity to PAS itself. The ferrous chelate, although not appreciably active, was much less toxic than the ferrous complex and is, in fact, relatively non-toxic. It was found to have an acute oral toxicity of over 2 Gm./Kg. in mice. The copper chelate, however, killed mice at a dose of 41.7 mg./Kg. but not at 20.8 mg./Kg. after intraperitoneal injection. Detailed results of this testing are presented in Table I.

TABLE I.—IN VIVO ANTITUBERCULAR ACTIVITIES OF THE PAS-METAL CHELATES^a

Compound	% in Diet	No of Mice	Mortality, %	Mean Survival Time in Days ^b
(PAS) ₂ Cu	0.5	5	0	23+
(PAS) ₂ Cu	0.01	5	20	22.8
INH ^c	0.01	10	0	23+
Control	.	10	60	21+
(PAS) ₂ Fe	0.1	5	60	20.6+
(PAS) ₂ Fe	0.02	5	80	20.6+
PAS ^c	0.5	5	0	21+
Control	.	10	80	20.3+
PAS ^c	0.5	10	30	20.2+
INH ^c	0.01	10	0	21+
Control	.	10	80	19.5+

^a Determined at the Lilly Research Laboratories by W. B. Sutton.

^b A + indicates that the surviving animals were sacrificed at the indicated times for examination of lesions.

^c PAS = *p*-aminosalicylic acid, INH = isonicotinic hydrazide.

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† Eli Lilly and Company Fellow, 1955-1956.

The authors are indebted to Drs. K. K. Chen and W. B. Sutton of the Lilly Research Laboratories for their cooperation in carrying out the antitubercular and toxicity tests.

The method of preparation of the PAS chelates followed that for the PAS complexes already described (2), except that the reactions were allowed to become acidic, thus allowing the isolation of the initially-formed dark colored precipitates. Evidence for chelation, according

to the criteria listed by Martell and Calvin (3), included: a marked drop in pH during the reaction, absence of metal ions in solution, decreased aqueous solubility, intensification of color, and analytical results in good agreement with a chelate structure having a 2:1 ratio of PAS to metal. Saturated aqueous solutions of the chelates gave negative tests for the metal ions using either ferrocyanide, ferrieyanide, or thiocyanate indicators. A positive test was given by the copper complex (I) with ferrocyanide ion, however. Suspensions of the chelates, after standing in dilute hydrochloric acid at a pH of 3.0 also gave negative tests for metal ions.

Elemental analyses were carried out on the air-dried products and the results are presented in Table II along with the corresponding information for the copper complex. It should be pointed

The ultraviolet absorption spectral characteristics of PAS and its copper derivatives were compared in an attempt to show further differences between the chelate and complex. The position of the maxima observed for PAS itself remained unchanged on formation of either the complex or chelate, but the intensity of absorption (per salicylate ring) increased slightly at 265 $m\mu$ and decreased slightly at 300 $m\mu$ (see Table III). Little difference in spectral characteristics was noted, however, between chelate and complex. The failure of chelate or complex formation to appreciably alter the position of the maxima has also been noted with the metal chelates of riboflavin (4).

Examination of another physical property, the oil-water partition coefficient, was made not only to discover differences between the chelate

TABLE II.—ANALYSES OF THE PAS-METAL CHELATES^a

Compound	Formula ^b	Analyses, %						H ₂ O, %	
		C	H	N	M	K. F. ^c			D ^d
(PAS) ₂ Cu complex	C ₁₄ H ₁₀ N ₂ O ₆ CuNa ₂ ·4H ₂ O	Calcd. 34.75	3.72	5.80	13.13				
		Found 34.94	3.55	5.80	14.89	15.45		14.89	15.15
(PAS) ₂ Cu chelate	C ₁₄ H ₁₂ N ₂ O ₆ Cu·H ₂ O	Calcd. 43.58	3.63	7.26	16.47			4.67	
		Found 43.48	3.80	7.37	15.81	4.85			5.25
(PAS) ₂ Fe chelate	C ₁₄ H ₁₂ N ₂ O ₆ Fe·4H ₂ O	Calcd. 38.92	4.63	..	12.93			16.67	
		Found 39.29	3.43	..	12.31				15.44

^a The carbon-hydrogen analyses were determined at the Weiler and Strauss Microanalytical Laboratory, Oxford, England. The nitrogen analyses were obtained by the Kjeldahl method.

^b The compounds were dried at room temperature by exposure to air to avoid loss of water of hydration as far as possible.

^c K. F. refers to the Karl Fischer determination.

^d D refers to vacuum drying at 100° over phosphorus pentoxide.

out that the variable water contents of these compounds, caused by variations in drying times and procedures, make it difficult to secure exact analytical values. The metal content was determined by ashing at 1,000°, and the water analysis was accomplished both by the Karl Fischer procedure and by loss of weight after vacuum drying at 100°. Unlike the PAS complexes, no sodium ion was present in the chelates. Although an alkaline reaction medium was employed in their preparation, the chelates were isolated at pH values of 4.0 and 5.7, respectively, for the copper and iron derivatives. These results permit the postulation of the following structure (II) for a divalent metal chelate of PAS.

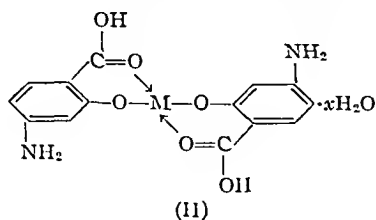


TABLE III.—ABSORPTION SPECTRAL CHARACTERISTICS

Compound	λ Max., $m\mu^a$	ϵ Max.
PAS	265	12,500
	300	9,075
(PAS) ₂ Cu complex	265	27,290
	300	17,470
(PAS) ₂ Cu chelate	265	26,490
	300	17,220
(PAS) ₂ Fe chelate	265	26,880
	300	18,320

^a The spectra were determined in water using a Beckman Model DU quartz spectrophotometer. Concentrations of approximately 10 mg. per liter were used.

and complex, but also to obtain information regarding the penetrability of the metal derivatives into the cells. Oleyl alcohol was selected for this purpose in preference to the more viscous vegetable oils, in accordance with its properties as described by Albert (5). It has also been shown by Collander (6) that the distribution of a series of substances between water and poorly miscible liquids always follows the same order,

although greater differences in partition coefficients within a given series are obtainable with the liquids of lower water solubility. The coefficients were obtained by measuring spectrophotometrically the percentage of compound extracted by oleyl alcohol from 10^{-5} to 10^{-6} *M* aqueous solutions. At this low concentration, the values observed can, of course, be no more than approximate. Reference to Table IV shows that the copper chelate is considerably more fat soluble (about thirty-fold) than the copper complex, as might be expected from their relative antitubercular activities. The fact that liposolubility is necessary, but is not alone a determinative factor in antitubercular activity, is shown by the comparable liposolubility of the ferrous to the cupric chelate and the relative lack of antitubercular properties by the ferrous chelate.

TABLE IV—OIL-WATER PARTITION COEFFICIENTS AT 25°

Compound	Concn, Aq Phase $\times 10^6$ <i>M</i>	pH Aq Phase	Partition Coeff $\times 10^3$ Oleyl Alc / H ₂ O
(PAS) ₂ Cu complex	5	9.8	8
(PAS) ₂ Cu chelate	6	5.2	244
(PAS) ₂ Fe chelate	10	4.7	174

It may be stated, on the basis of the evidence presented here and elsewhere (1), that PAS probably exerts its antitubercular action as a cupric chelate. Both *in vitro* and *in vivo* tests have shown the copper derivative to be equal to or superior to PAS itself. That the cupric chelate and not the cupric complex is successful against experimental tuberculosis is undoubtedly due to the much greater lipophilic character of the chelate which allows greater penetration of the cells. The increased *in vitro* activity of both PAS and the copper complex in the presence of excess copper ion points to the possibility that a 1:1 chelate may be the important species, and the fact that doses of the copper chelate comparable to those of PAS were effective in mice presents a strong likelihood that a chelate is the active antitubercular form of this agent. While the chelates were too insoluble to permit the determination of stability constants by customary methods, their stability in water at a pH of 3.0 over prolonged periods would indicate an ability to remain intact in the body. The failure of the ferrous chelate to show appreciable *in vivo* antitubercular activity, whereas both the cupric and ferrous

chelates of 8-hydroxyquinoline are known to be the active bacteriostatic forms of this drug (7), and its comparable liposolubility to the cupric chelate, indicate a high degree of selective toxic action in the antitubercular activity of *p*-amino salicylic acid chelates.

EXPERIMENTAL

Preparation of the Metal Chelates.—An aqueous solution of copper sulfate (0.05 mole) was added slowly with stirring to a solution of sodium *p*-aminosalicylate (0.1 mole) in 100 ml of water. The pH dropped from 9.5 to 4.0, as determined by a Beckman pH meter, and a green-black precipitate formed immediately. The suspension was stirred for an hour, filtered, and the product was washed free of sulfate ion with cold water. The dark green product was allowed to dry by exposure to air at room temperature, and a yield of 95% was obtained, based on the formula proposed for the copper chelate in Table II.

The ferrous chelate was prepared in identical fashion, using 0.025 mole of ferrous sulfate and 0.05 mole of sodium *p*-aminosalicylate. The pH dropped from 9.5 to 5.7 during this reaction. A dark red-brown precipitate was isolated in 48% yield, based on the formula in Table II. This compound was appreciably more water-soluble than the copper chelate, and a sizable portion was lost during the washing.

Analysis of the Metal Chelates.—The metal content of the chelates was determined by ashing to constant weight at 1,000°. The ash from the copper chelate was weighed as cupric oxide and that from the ferrous chelate as ferric oxide, and the results were reproducible. The copper complex could not be analyzed by this method because of the presence of sodium carbonate in the ash, so the copper was determined by the standard iodometric procedure. The usual method for analyzing ferrous ion by dichromate oxidation could not be used for the ferrous chelate, since the PAS interfered and no endpoint was visible.

Analysis of the water content was accomplished by the Karl Fischer method using platinum electrodes and a Beckman pH meter. Vacuum drying (10–20 mm) to constant weight at 100° over phosphorus pentoxide gave comparable values for water to those obtained from the Karl Fischer procedure, but less drastic drying conditions gave variable results.

The chelates were found to be stable at a pH of 3.0 by suspending the chelates in dilute hydrochloric acid and testing for metal ion with nonchelating indicators. Negative tests for metal ion were obtained with each of the chelates using ferrocyanide, ferriyanide, and thiocyanate ions after the chelates remained in contact with the acid overnight. A positive test for cupric ion was found with the copper complex, however.

Measurement of Partition Coefficients.—Commercial oleyl alcohol (DuPont Co., b.p. 137–142°/2 mm) was used to extract the complexes from water at 25°. Approximately 5 mg of complex was equilibrated between the aqueous and organic phases by stirring the suspensions for three hours,

by which time all the solid had dissolved. A liter of water was required to dissolve these amounts, giving 10^{-5} – 10^{-6} *M* aqueous solutions of chelate or complex, and an equal volume of oleyl alcohol was used. After separation of the layers, residual droplets of oil were removed by filtration through cotton. The concentration of the compound in the aqueous phase was determined spectrophotometrically, and the percentage of compound in each phase was calculated.

REFERENCES

- (1) Roth, W., Zuber, F., Sorkin, E., and Erlenmeyer, H., *Helv. Chim. Acta*, **34**, 430(1951)
- (2) Foye, W. O., *THIS JOURNAL*, **44**, 415(1955).
- (3) Martell, A. E., and Calvin, M., "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, pp 19–75
- (4) Mahler, H. R., Fairhurst, A. S., and Mackler, B. J., *Am. Chem. Soc.*, **77**, 1514(1955).
- (5) Albert, A., and Hampton, A., *J. Chem. Soc.*, 1954, 505.
- (6) Collander, R., *Acta physiol. Scand.*, **13**, 363(1947)
- (7) Albert, A., Gibson, M. I., and Rubbo, S. D., *Brit. J. Exptl. Pathol.*, **34**, 119(1953)

Metal Chelates and Antitubercular Activity IV.*

Isonicotinyl Hydrazide

By WILLIAM O. FOYE and RONALD N. DUVAL†

Metal chelates of isonicotinyl hydrazide (INH) containing divalent copper, cobalt, iron, and zinc and having both 1:1 and 2:1 ratios of INH to metal have been isolated and characterized. These chelates showed *in vivo* antitubercular activities in mice comparable to that from INH itself. The 2:1 chelates were found to be considerably more lipophilic (fifty to ninety-fold) than the 1:1 chelates, but underwent conversion to 1:1 chelates at equilibrium. Toxicity determinations on the chelates showed them to resemble the corresponding inorganic salts in their toxic manifestations in mice. These findings suggest that the INH chelates are present mainly as the 1:1 species *in vivo*, and permit the postulation that INH exerts its antitubercular action as a 1:1 metal chelate.

A TENFOLD INCREASE in the tuberculostatic action of isonicotinyl hydrazide (INH) in the presence of cupric ion was reported by Erlenmeyer, *et al.* (1), on the basis of *in vitro* tests. It was also shown that INH was capable of chelating copper ion giving a 1:1 ratio of INH to copper, and it was suggested that chelation of heavy metal ions played an important part in the antitubercular action of this drug. A chelate structure having a 2:1 ratio of INH to copper was also postulated by Fallab and Erlenmeyer (2), and Albert (3), as well, showed that both 2:1 and 1:1 chelates of INH were capable of existence. Albert also revealed that other hydrazides have equal or greater affinities for metal ions than has INH, but they generally show much lower antitubercular effects (4).

Cymerman-Craig and Rubbo (5) have also presented evidence that INH exerts its tuberculostatic action by means of a metal ion chela-

tion. This was accomplished by blocking the ability of INH to chelate and showing loss of antitubercular activity *in vitro*. These investigators later tested the 1:1 cupric chelate of INH *in vivo* using an ulcer healing technique in mice, and described the chelate to be as active as INH or any of its derivatives, although it showed both local and systemic toxicities (6). They believed the copper chelate to be the active form of the drug *in vivo*.

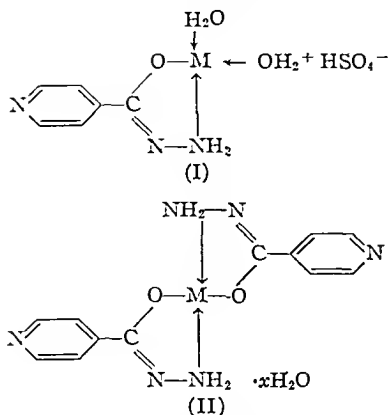
In this communication, the preparation of several divalent metal chelates of INH and their *in vivo* antitubercular activities are reported. Chelates having a 1:1 ratio of INH to metal (I) were isolated from acidic media, and chelates having a 2:1 ratio (II) were isolated on the alkaline side of neutrality. Coprecipitation of metal hydroxide in the latter reaction was avoided by keeping the pH from exceeding 7.5. The usual indications of chelation were noted, including a marked drop in pH, considerable decrease in aqueous solubility, formation of colored products, and elemental analyses which agree with theoretical values for chelate structures. Chelates (1:1), with cupric, ferrous, and zinc ions, and 2:1 chelates with cupric and cobaltous ions were isolated, and the analytical results are recorded in Table I. An insoluble 1:1 cobalt chelate could not be isolated.

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When these chelates were fed to mice infected with *M. tuberculosis* H37Rv, as previously described for complexes of *p*-aminosalicylic acid (7), their activities were comparable to that of INH itself. Comparison of the relative survival times and mortality percentages of mice protected by the chelates with those of INH-fed mice and unprotected controls would indicate the chelates to be as effective as INH. This becomes evident on comparing the amounts of INH present in the chelates with the amount of uncombined INH used as comparison standard;

in Table III. Comparison of these toxicities with those from corresponding amounts of the inorganic salts shows close similarities. Other biological effects of the chelates, such as decrease of activity of the mice, decrease of respiration,

TABLE II.—IN VIVO ANTITUBERCULAR ACTIVITIES OF THE INH-METAL CHELATES^a

Chelate	Diet, %	No. of Mice	Mortality, %	Mean Survival Time, days ^b
INH-Cu ⁺ HSO ₄ ⁻	0.5	5	0	21+
	0.2	5	0	21+
	0.1	5	0	21+
	0.02	5	0	21+
	0.01	5	20	20.9
(INH) ₂ Cu	0.1	5	0	21+
	0.02	5	0	21+
	0.1	5	0	21+
(INH) ₂ Co	0.1	5	0	21+
	0.02	5	0	21+
	0.1	5	0	21+
INH-Zn ⁺ HSO ₄ ⁻	0.2	5	0	21+
	0.1	5	0	21+
	0.02	5	0	21+
INH-Fe ⁺ HSO ₄ ⁻	0.1	5	0	21+
	0.02	5	0	21+
	0.1	5	0	21+
INH	0.01	5	0	21+
Controls		10	80	20.3+

^a Determined at the Lilly Research Laboratories by W. B. Sutton.

^b A+ indicates that the surviving animals were sacrificed at the indicated times for examination of lesions.

TABLE I.—ANALYSES OF THE INH-METAL CHELATES^a

Chelate	Formula ^b		C	H	% S	M	K. F. ^c	H ₂ O, %	D ^d
INH-Cu ⁺ HSO ₄ ⁻	C ₆ H ₇ N ₃ O ₅ SCu·2H ₂ O	Calcd.	21.65	3.31	9.62	19.10		10.82	
		Found	22.00	3.25	9.07	18.25	11.47		11.97
(INH) ₂ Cu	C ₁₂ H ₁₂ N ₆ O ₂ Cu·3H ₂ O	Calcd.	36.97	4.62		16.30		13.86	
		Found	37.21	3.92		18.03			14.57
(INH) ₂ Co	C ₁₂ H ₁₂ N ₆ O ₂ Co·4H ₂ O	Calcd.	35.71	4.96		14.62		17.86	
		Found	35.81	3.89		14.44	13.90		14.09
INH-Zn ⁺ HSO ₄ ⁻	C ₆ H ₇ N ₃ O ₅ SZn·2H ₂ O	Calcd.	21.52	3.29	9.57	19.54		10.76	
		Found	21.58	2.96	9.22	18.24	13.42		12.44
INH-Fe ⁺ HSO ₄ ⁻	C ₆ H ₇ N ₃ O ₅ SFe·2H ₂ O	Calcd.	22.15	3.38	9.85	17.18		11.08	
		Found	21.85	3.15	9.27	15.93	12.08		10.14

^a The carbon-hydrogen analyses were determined at the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

^b The compounds were dried at room temperature by exposure to air to avoid loss of water of hydration as far as possible.

^c K. F. refers to the Karl Fischer determination.

^d D refers to vacuum drying at 100° over phosphorus pentoxide.

since 0.025% of the 1:1 copper chelate, for instance, should give the same effect as the 0.01% INH used as standard, based on molecular weights. Histological examination of the mice, however, revealed that a 0.5% dosage level of the 1:1 copper chelate was less effective than a 0.01% level of INH. Detailed results of this testing, carried out at the Lilly Research Laboratories, are shown in Table II.

Determination of toxicities of the INH chelates in mice was also done at the Lilly Research Laboratories, and the results are recorded

skin color, and abdominal tone loss, were also closely paralleled by the injection of the corresponding metal ions as salts. These results indicate that the chelates are decomposed to some extent *in vivo*, and that metal ions are liberated and produce toxic effects. However, the 1:1 chelates are more toxic than would be expected from their metal contents. Whether this decomposition occurs before or after the antitubercular action of the chelate is, of course, not evident from this method of examination. Albert (3) has measured the stability constants of INH-metal

TABLE III.—TOXICITIES OF THE INH-METAL CHELATES^a

Compound	Dose, I. P., mg./Kg., in Mice ^b						
	10	25	50	100	250	500	1,000
INH-Cu ⁺ HSO ₄ ⁻ ·2H ₂ O	..	3-4 days	5 hr.	1 hr.
(INH) ₂ Cu·3H ₂ O	1-2 days	3 hr.	23 min.- 1 day
CuSO ₄ ·5H ₂ O	2 days	40 min.- 1 day	1-1½ hr.	40 min.
INH-Fe ⁺ HSO ₄ ⁻ ·2H ₂ O	1 day	3 hr.	2 hr.
FeSO ₄ ·7H ₂ O	3 days	2 days	4 hr.
(INH) ₂ Co·4H ₂ O	1 day	..
Co(NO ₃) ₂ ·6H ₂ O	1 day	9 min.- 1 day	4-6 min.
INH-Zn ⁺ HSO ₄ ⁻ ·2H ₂ O	1-2 days
ZnSO ₄ ·7H ₂ O	5 days	17 min.- 2 hr.	17-19 min.	..

^a Determined at the Lilly Research Laboratories by R. C. Rathhunn.^b Generally three mice were used for each determination. The times recorded indicate that required for death.

chelates and shown their stabilities to be only slightly less than those of the common amino acid chelates, which chelates are considered to be capable of existence *in vivo* (8, 9).

The ultraviolet absorption spectral characteristics of INH and the divalent metal chelates prepared are listed in Table IV. Little change in

TABLE IV.—ABSORPTION SPECTRAL CHARACTERISTICS

Compound	λ Max., $m\mu^a$	ϵ Max.
INH	264	4,780
(INH) ₂ Co	265	7,660
(INH) ₂ Cu	265	6,380
INH-Cu ⁺ HSO ₄ ⁻	265	3,920
INH-Zn ⁺ HSO ₄ ⁻	262.5	4,520
INH-Fe ⁺ HSO ₄ ⁻	262	6,070

^a The spectra were determined in water at concentrations of 10 mg. per liter.

the position of maximum absorption for INH took place on formation of the chelates, as was previously observed for the chelates of *p*-aminosalicylic acid (10); but the intensity of absorption decreased with each chelate except the ferrous, when compared with the value to be expected from the amount of INH present. The 2:1 chelates could be distinguished from the 1:1 chelates by the nature of the absorption curves, however. The 2:1 chelates showed pronounced minima at 243-245 $m\mu$, whereas the 1:1 chelates gave flatter curves with no pronounced minima. Prolonged stirring (three to four hours) was necessary to dissolve appreciable amounts of most of these chelates in water, but no attempt was made to reach equilibrium conditions. Essentially the same characteristics were found for the zinc chelate which dissolved at once.

Examination of the oil-water partition coefficients (Table V) of the INH chelates shows the 2:1 chelates to be fifty to ninety-fold more fat

TABLE V.—OIL-WATER PARTITION COEFFICIENTS, 25°

Chelate	Concn., Aq. Phase $\times 10^5 M$	pH Aq. Phase	Partition Coeff., Oleyl Alc./H ₂ O
INH-Cu ⁺ HSO ₄ ⁻	15	4.9	0.109
INH-Fe ⁺ HSO ₄ ⁻	15	5.1	0.155
INH-Zn ⁺ HSO ₄ ⁻	15	6.3	0.144
(INH) ₂ Cu	0.75	6.3	10.11
(INH) ₂ Co	1.1	5.8	5.54

soluble than the 1:1 chelates at the concentrations measured. Oleyl alcohol was used for this determination, in accordance with the advantages outlined by Albert (11). The coefficients were obtained by measuring spectrophotometrically the percentage of compound extracted by oleyl alcohol from 10⁻⁵ *M* aqueous solutions when freshly prepared. An attempt to reach equilibrium conditions is shown in Table VI. A gradual conversion to an equilibrium, expected to exist between INH and the 1:1 and 2:1 chelates in accordance with Albert's stability constants (3) was apparent after two weeks, with most of the chelate present as the 1:1 species.

TABLE VI.—OIL-WATER PARTITION COEFFICIENTS AFTER TWO WEEKS, 25°

Chelate	Concn., Aq. Phase $\times 10^5 M$	pH Aq. Phase	Time	Partition Coeff., Oleyl Alc./H ₂ O
INH-Cu ⁺ HSO ₄ ⁻	15	4.9	3 hr.	0.109
	15	4.9	2 wks.	0.088
	3.0	6.1	3 days	0.134
	3.0	6.1	2 wks.	0.089
(INH) ₂ Cu	0.75	6.3	3 hr.	10.11
	0.75	6.3	1 wk.	0.222
	0.75	6.3	12 days	0.285
	0.75	6.3	2 wks.	0.167

The antitubercular testing results also indicated a similar shift to 1:1 chelate in the animal body, since the same relative activity was shown by the 2:1 as by the 1:1 chelates.

It is apparent from the much greater lipophilic character of the 2:1 chelates that they should penetrate the cells more readily than the 1:1 chelates (12). Albert (11) has previously demonstrated, for instance, that lowering the liposolubility of 8-hydroxyquinoline analogs and presumably that of their metal chelates as well, considerably reduced or removed their antibacterial activity. In the present case, no difference in antitubercular activity was noted between the 1:1 and 2:1 chelates and INH itself, which in addition to the toxic effects, suggests that the 2:1 chelates are undergoing conversion mainly to 1:1 chelates. In fact, it is doubtful that any appreciable amount of 2:1 chelate would be produced in the body after administration of free INH due to the necessity for alkaline conditions for its formation, and the direction which the equilibrium takes in neutral or acid media. In view of the previous findings regarding the tuberculostatic action of INH chelates (1, 4, 6), it appears from the evidence presented here that INH tends to act as a 1:1 metal chelate, since otherwise chelate formation would be expected to reduce the antitubercular activity of INH, and both the 2:1 chelate and free INH should be readily converted to the 1:1 chelate under biological conditions.

EXPERIMENTAL

Preparation of the Metal Chelates.—The 1:1 chelates were prepared by adding an aqueous solution of the metal sulfate (0.02 mole) slowly with stirring to 2.7 Gm. (0.02 mole) of isonicotinyl hydrazide¹ in 50 ml. of water. Precipitation took place within a few minutes, and the pH dropped from 7.1 to 3.2 during the addition of copper sulfate, from 7.1 to 5.9 using zinc sulfate, and from 7.2 to 4.8 with ferrous sulfate. The mixtures were stirred for one hour, filtered, and washed with water. The products were dried by exposure to air at room temperature. A 92% yield of pale green copper chelate, an 83% yield of white zinc chelate, and an 87% yield of bright orange ferrous chelate were isolated, based on the formulas proposed in Table I.

The 2:1 chelates were obtained by the dropwise addition of aqueous solutions of copper sulfate and cobaltous chloride (0.01 mole) to 0.02 mole of isonicotinyl hydrazide¹ in 50 ml. of water while maintaining the pH at approximately 7.5. After being stirred for an hour, the mixtures were filtered, washed with water, and air dried. An 83% yield of green copper chelate and a 72% yield of pink

cobalt chelate were isolated. With both the 1:1 and 2:1 chelates, no further purification was done prior to analysis in order to avoid loss of water of hydration and avoid, as a result, products of varying composition.

Analysis of the Metal Chelates.—Metal analyses were done by ashing at 1,000° as reported earlier (10). Copper was also determined by the standard iodometric procedure. Sulfur was determined by dissolving the 1:1 chelates in hydrochloric acid T.S. and precipitating the sulfate as barium sulfate. Water of hydration was measured both by the Karl Fischer method and by vacuum drying at 100° over phosphorus pentoxide as previously described (10).

Qualitative tests for metal ion were made on both the saturated aqueous solutions of the chelates and suspensions in dilute hydrochloric acid at a pH of 3.0 after an hour. The two copper chelates gave negative tests in both cases using ferrocyanide and ferricyanide indicators. The ferrous chelate showed positive tests for ferrous ion in both media, as did the cobalt and zinc chelates. In no case was a greater intensity of color produced from the suspensions in dilute hydrochloric acid after an hour than from the aqueous solutions, however. These indications are in agreement with the stability constants of INH-metal chelates determined by Albert (3).

Measurement of Partition Coefficients.—Oleyl alcohol (DuPont Co., b. p. 137–142°/2 mm.) was used to extract the chelates from 10^{-4} to 10^{-6} M aqueous solutions as previously described (10). The use of buffers to provide a uniform pH in the aqueous phase was considered inadvisable, since change of pH obviously alters the ligand-metal ratio, and many buffer systems are capable of undergoing salt or complex formation with metal ions or chelates. In the case of the equilibrium determinations, the 1:1 chelate showed sufficient solubility in the aqueous phase to permit determinations to be made at two concentrations. For the 2:1 chelate however, values for only one concentration are reported, since at higher concentrations, the solubility limit in oleyl alcohol was exceeded, and at lower concentrations, the accuracy of measurement by the spectrophotometer used (Beckman Model DU) is questionable.

Determination of Antitubercular Activities.—Mice infected with *M. tuberculosis* H37Rv were fed the chelates as previously reported (7), and the mean survival times were compared with those of control mice receiving either no drug or INH as standard.

REFERENCES

- (1) Sorkin, E., Roth, W., and Erlenmeyer, H., *Helv. Chim. Acta*, **35**, 1736 (1952).
- (2) Fallab, S., and Erlenmeyer, H., *Experientia*, **8**, 298 (1952).
- (3) Albert, A., *ibid.*, **9**, 370 (1953).
- (4) Albert, A., *Nature*, **177**, 525 (1956).
- (5) Cymerman-Craig, J., Willis, D., Rubbo, S. D., and Edgar, J., *ibid.*, **176**, 34 (1955).
- (6) Rubbo, S. D., Edgar, J., Cymerman-Craig, J., Vaughan, G. N., and Willis, D., *ibid.*, **177**, 480 (1956).
- (7) Foye, W. O., *This Journal*, **44**, 415 (1955).
- (8) Albert, A., *Biochem. J.*, **47**, 531 (1950); **50**, 690 (1952).
- (9) Fallab, S., and Erlenmeyer, H., *Experientia*, **11**, 174 (1955).
- (10) Foye, W. O., and Duvall, R. N., *This Journal*, **47**, 282 (1958).
- (11) Albert, A., and Hampton, A., *J. Chem. Soc.*, **1954**, 505.
- (12) Davson, H., and Danielli, J., "The Permeability of Natural Membranes," Cambridge University Press, Cambridge, Eng., 1943, p. 108.

¹ A gift of the Massachusetts General Hospital.

Interaction of Preservatives with Macromolecules I.

Binding of Parahydroxybenzoic Acid Esters by Polyoxyethylene 20 Sorbitan Monooleate (Tween 80)*

By N. K. PATEL and H. B. KOSTENBAUDER

A quantitative evaluation of the degree of intermolecular association between *p*-hydroxybenzoic acid esters and Tween® 80 was obtained by means of an equilibrium dialysis study employing a semipermeable nylon membrane. Data obtained facilitate calculation of the quantity of preservative which must be added to an aqueous system containing a known concentration of Tween® 80 in order to have the desired concentration of unbound preservative.

NUMEROUS REPORTS have appeared in recent years concerning the inactivation of various preservatives in the presence of several non-ionic surfactants (1-9) and vegetable gums (10-12) commonly employed in pharmaceutical systems. One of the examples which has most frequently been cited is that of the inhibition of phenolic preservatives in the presence of surfactants which are polyether derivatives of fatty acid esters. Several investigators have suggested that this phenomenon can probably be attributed to the formation of a complex between the preservative and the surfactant (8, 9).

As demonstrated by Allawala and Riegelman (13, 14) in their studies of iodine solubilized by surface-active agents, it is the thermodynamic activity rather than the stoichiometric concentration of the toxic substance which is related to the antimicrobial activity. Therefore, in the presence of an agent which is capable of forming a complex with the preservative, it is to be expected that both the thermodynamic and antimicrobial activity will be diminished. The present investigation was undertaken to obtain a quantitative evaluation of the association between *p*-hydroxybenzoic acid esters and Tween® 80¹, for the purpose of relating this possible interaction to the reported inhibition of the preservative.

Higuchi and Lach (15) and Guttman and Higuchi (16) demonstrated the tendency of phenols to form molecular complexes with polyethers such as the polyethylene glycols. It would thus be expected that phenolic preservatives such as the *p*-hydroxybenzoates might form molecular complexes with the ethereal oxygen of

the polyoxyethylene groups of the Tween molecule.

A convenient method for investigating a system of this type is an equilibrium dialysis technique which utilizes a membrane that permits free passage of the *p*-hydroxybenzoate, but is impermeable to the macromolecule. At equilibrium the activity of the *p*-hydroxybenzoate will be identical on both sides of the membrane, and for reasonably dilute solutions it may be assumed that the concentration of free *p*-hydroxybenzoate on both sides of the membrane will be essentially equal. Thus, by placing the macromolecule on one side of the membrane and determining the concentration of *p*-hydroxybenzoate on the opposite side of the membrane, it is possible to determine the concentration of free or unbound preservative in equilibrium with the macromolecule. It is to be expected that the effectiveness of the preservative in the presence of the macromolecule might parallel the concentration of free *p*-hydroxybenzoate. It should perhaps be noted that the dialysis method permits an estimation of the degree of binding should the association be due to the postulated complex formation or to some other mechanism such as preferential solubility of the preservative within a micelle.

EXPERIMENTAL

Reagents.—Recrystallized methyl *p*-hydroxybenzoate,² m. p. 125–126°; recrystallized propyl *p*-hydroxybenzoate,³ m. p. 94–95°; Tween® 80, a commercial sample.

Selection of the Dialysis Membrane.—Bags fashioned from Visking cellulose casing were originally investigated for use as dialysis membranes; but they were found to be unsatisfactory, since they were not impermeable to the Tween® The

* Received August 9, 1957, from the School of Pharmacy, Temple University, Philadelphia, Pa.

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¹ Tween® 80 is polyoxyethylene 20 sorbitan monooleate (Atlas Powder Co., Wilmington, Del.).

² Methyl Parasept®, Purified, supplied through the courtesy of Heyden Newport Chemical Corporation, New York.

³ Propyl Parasept®, Purified, supplied through the courtesy of Heyden Newport Chemical Corporation, New York.

presence of Tween® on both sides of the membrane was readily detected both by the foaming tendency of the solution upon agitation, and by the white, oily precipitate formed when a small quantity of the Tween® solution was added to a 5% aqueous solution of phenol.

A thin nylon membrane⁴ was found to be highly satisfactory, since it proved to be permeable to the *p*-hydroxybenzoate and impermeable to the macromolecule. In the course of the investigation it was found that the nylon membrane had a tendency to bind the *p*-hydroxybenzoate, but this binding was readily accounted for in the calculations.

Dialysis Method.—The dialysis membrane was employed in the form of small nylon sacks which were made as uniform in size as possible. Into each bag was placed 20 ml. of an aqueous solution of the preservative, and the bag was then tightly closed by knotting the open end. Each nylon bag was then placed in a wide-mouth, ground-glass stoppered bottle containing 20 ml. of the Tween® 80 solution. The bottles were then stoppered tightly and agitated for fifteen hours at 30°. Where very high concentrations of paraben were desired it was necessary to add additional solid paraben to the external solution.

Samples containing only purified water as the external solution were also included to verify attainment of equilibrium and to provide data on the binding of the preservative by the nylon membrane.

After equilibration, samples were removed from the external solution and the concentration of *p*-hydroxybenzoate was determined spectrophotometrically at a wavelength of 255 mμ. It was found that the concentration of *p*-hydroxybenzoate outside the bag could be satisfactorily determined either by a direct spectrophotometric analysis or by calculation after correcting for the amount of paraben bound by the nylon.

Solubility Method.—The solubility of methyl *p*-hydroxybenzoate was studied by placing an excess of the ester (0.25 Gm.) in rubber capped vials containing 10 ml. of Tween® 80 solution. The vials were then agitated for forty-eight hours at 27°, and the concentration of methyl *p*-hydroxybenzoate in solution was determined spectrophotometrically. It was found that the Tween® did not interfere with the spectrophotometric assay. The samples were diluted with water many fold before the spectrophotometric determinations were performed, and the absorption curve for the paraben in these extremely dilute Tween® solutions was identical to that in distilled water.

RESULTS AND DISCUSSION

Binding of Methyl *p*-Hydroxybenzoate by Nylon.

—In the course of the investigation it was found that even when the system consisted only of the *p*-hydroxybenzoate, purified water, and the nylon membrane, the total quantity of *p*-hydroxybenzoate found in solution at equilibrium was less than that added to the system. This discrepancy was attributed to binding of the *p*-hydroxybenzoate by the nylon membrane and was found to be a reversible binding which is dependent on both the size of

the nylon membrane and the concentration of free paraben. By keeping the size of the dialysis bags constant throughout the experiment, the *p*-hydroxybenzoate bound by the nylon was a function only of the concentration of free *p*-hydroxybenzoate. These data are illustrated in Fig. 1.

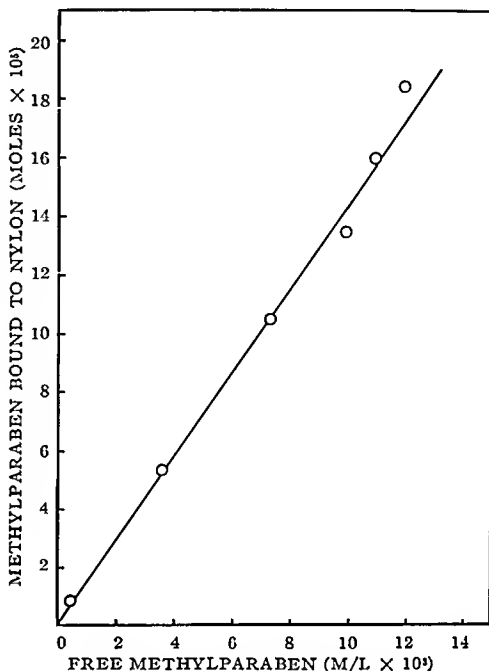


Fig. 1.—Binding of methyl *p*-hydroxybenzoate by the nylon membrane as a function of the concentration of free methyl *p*-hydroxybenzoate in water at 30°.

The binding of the paraben by the nylon is not unusual when the protein-like nature of the nylon is considered. Kostenbauder and Higuchi (17, 18) demonstrated that amides form molecular complexes in aqueous solution with certain organic compounds which act as proton donors, and it is probable that a similar interaction occurs between the phenolic preservative and the amide groups of the nylon.

The possibility of the surfactant being bound by the nylon membrane was not investigated, since it was assumed that any Tween® bound by the nylon would be negligible in comparison to the relatively high concentrations of Tween® added to the system. The validity of this assumption was confirmed by the solubility studies.

Dialysis Study of the Interaction Between Methyl *p*-Hydroxybenzoate and Tween® 80.—The degree of binding of methyl *p*-hydroxybenzoate by Tween® 80 was determined as the difference between the total amount of *p*-hydroxybenzoate added to the system and the *p*-hydroxybenzoate found at equilibrium as free *p*-hydroxybenzoate and *p*-hydroxybenzoate bound to the nylon membrane. The concentration of free *p*-hydroxybenzoate was determined spectrophotometrically in the internal

⁴ Supplied through the courtesy of Youngs Rubber Corporation, New York.

solution, and the *p*-hydroxybenzoate bound to the nylon was determined from Fig. 1.

Let P_t = total *p*-hydroxybenzoate added, P_b = *p*-hydroxybenzoate bound to Tween®, P_n = *p*-hydroxybenzoate bound to nylon, P_f = free *p*-hydroxybenzoate inside = free *p*-hydroxybenzoate outside, then $P_b = P_t - (2P_f + P_n)$.

An alternative method is to determine directly the concentration of *p*-hydroxybenzoate in solution outside the bag and the concentration of free *p*-hydroxybenzoate inside the bag. Then the *p*-hydroxybenzoate bound by the Tween® is the difference between the concentration outside and the concentration inside. The two methods were in excellent agreement over the concentration range employed.

Figure 2 shows the binding of methyl *p*-hydroxybenzoate by Tween® 80 as a function of both Tween® 80 concentration and free methyl *p*-hydroxybenzoate concentration. Figure 3 indicates

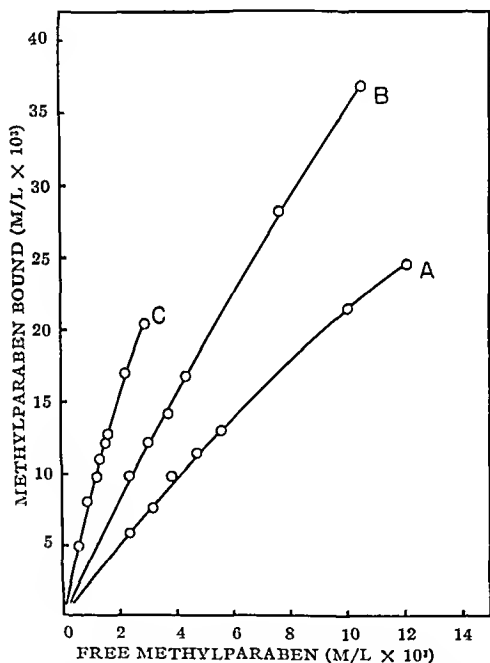


Fig. 2.—Binding of methyl *p*-hydroxybenzoate by Tween® 80 at 30°. A—3% (W/V) Tween® 80; B—5% (W/V) Tween® 80; C—10% (W/V) Tween® 80.

that the ratio, r , of total *p*-hydroxybenzoate in solution to the concentration of free *p*-hydroxybenzoate is a function of the concentration of Tween® 80. It is evident from these data that there is considerable interaction occurring between the preservative and the surfactant. At a concentration of 5% Tween® 80 only 22% of the total paraben present exists as free paraben, and at a concentration of 10% Tween® 80 only 12% of the total paraben exists as free paraben. The data illustrated in Fig. 3 facili-

tate the determination of the quantity of preservative which must be added to a system containing a known concentration of Tween® 80 in order to have the desired concentration of free preservative. Multiplying the desired concentration of free methyl *p*-hydroxybenzoate by the appropriate r value gives the concentration of total methyl *p*-hydroxybenzoate which must be employed.

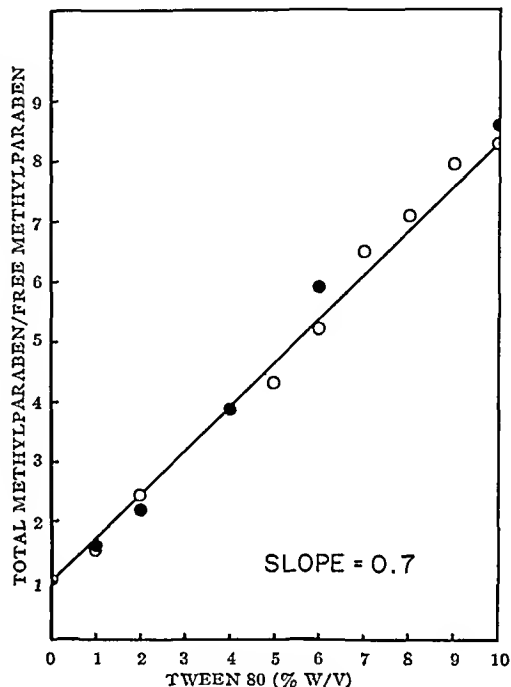


Fig. 3.—A plot showing the ratio, r , of total methyl *p*-hydroxybenzoate to free methyl *p*-hydroxybenzoate, at 30°, in aqueous solutions containing varying concentrations of Tween® 80. ○—Concentration of methyl *p*-hydroxybenzoate on each side of the membrane would be 3.84×10^{-3} molar in absence of Tween® 80; ●—concentration on each side of membrane would be 6.24×10^{-3} molar in absence of Tween® 80.

Figure 4 is a Langmuir-type plot of the binding of methyl *p*-hydroxybenzoate by three and five % solutions of Tween® 80. The data are plotted in this manner to obtain the limiting binding capacity of the Tween® unit at infinitely high methyl *p*-hydroxybenzoate concentration. The Y-intercept is the reciprocal of the limiting quantity of methyl *p*-hydroxybenzoate bound per gram of Tween® 80. This graph is of particular interest in the present investigation, since the existence of the Y-intercept suggests that complex formation is a factor in the binding of the preservative by the macromolecule. If the solc interaction in the system had been a partitioning of the preservative into a micelle, the expected curve would be a straight line through the origin.

The Y-intercept in Fig. 4 indicates that the limit-

ing binding capacity of the Tween® 80 is approximately 0.004 moles of methyl *p*-hydroxybenzoate per gram of Tween® 80. Using the method described by Schönfeldt (19, 20), and employing Polyethylene Glycol 4000⁶ and Polyethylene Glycol 6000⁶ as standards, the polyoxyethylene content of

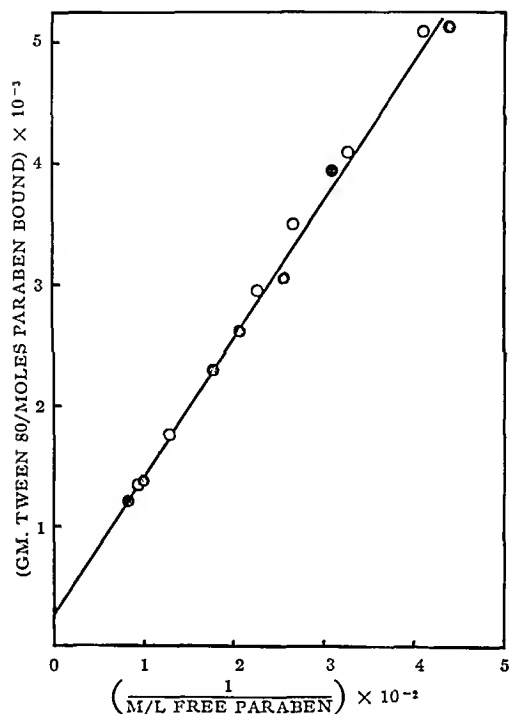


Fig. 4.—Langmuir-type plot to determine the limiting quantity of methyl *p*-hydroxybenzoate bound by Tween® 80 in infinitely high methyl *p*-hydroxybenzoate concentration. ●—3% (W/V) Tween® 80; ○—5% (W/V) Tween® 80.

the Tween® 80 was approximated as 0.016 polyoxyethylene groups per gram of Tween® 80. These results would correspond to a limiting ratio of 1 mole of bound methyl *p*-hydroxybenzoate for each 4 polyoxyethylene units of the Tween® 80. All data were obtained using a single lot of Tween® 80.

Dialysis Study of the Interaction between Propyl *p*-Hydroxybenzoate and Tween® 80.—Figure 5 shows the binding of propyl *p*-hydroxybenzoate as a function of Tween® 80 concentration. The interaction of the propyl ester with nylon was not studied; therefore, the degree of binding by the Tween® was determined by direct analysis of the solution on both sides of the membrane. Comparison of Figs. 3 and 5 indicates that propyl *p*-hydroxybenzoate has greater affinity for Tween® 80 than has the methyl ester. At a concentration of 5% Tween® 80 only 4.5% of the total propyl *p*-hydroxybenzoate was found to exist in the unbound form.

Solubility Study.—Figure 6 illustrates the solubility of methyl *p*-hydroxybenzoate as a function of

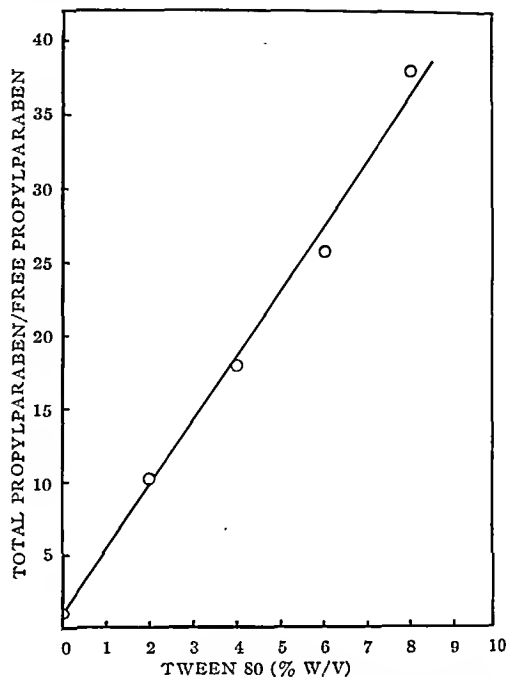


Fig. 5.—Ratio, *r*, of total propyl *p*-hydroxybenzoate to free propyl *p*-hydroxybenzoate, at 30°, as a function of Tween® 80 concentration. Concentrations of free propyl *p*-hydroxybenzoate in this study were within the range of 1.58×10^{-3} molar at 2% Tween® 80 to 0.50×10^{-3} molar at 8% Tween® 80.

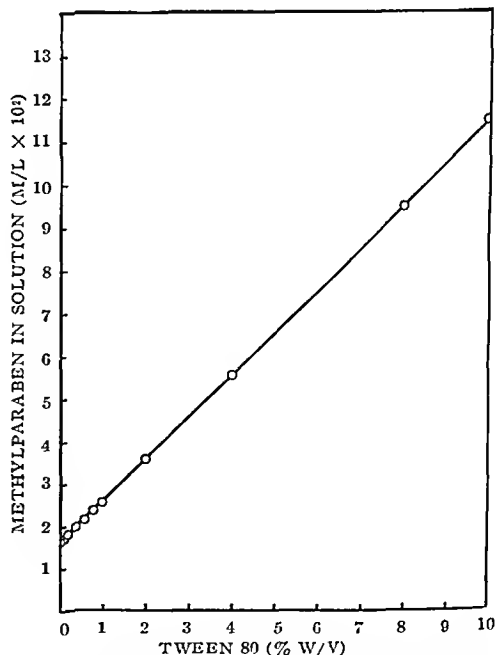


Fig. 6.—Solubility of methyl *p*-hydroxybenzoate in water at 27° as a function of Tween® 80 concentration.

⁶ Carbowax® 4000 and Carbowax® 6000, respectively, Union Carbide Chemicals Co., New York.

Tween® 80 concentration The solubility determinations were made at a temperature of 27°, since at 30° a saturated solution of methyl *p*-hydroxybenzoate in some concentrations of Tween® 80 resulted in the formation of a white, oily precipitate (21) which was not readily filterable

Figure 7 was obtained by treating the solubility data in the same manner as the dialysis data. In the solubility study, the free *p*-hydroxybenzoate concentration was a constant corresponding to the

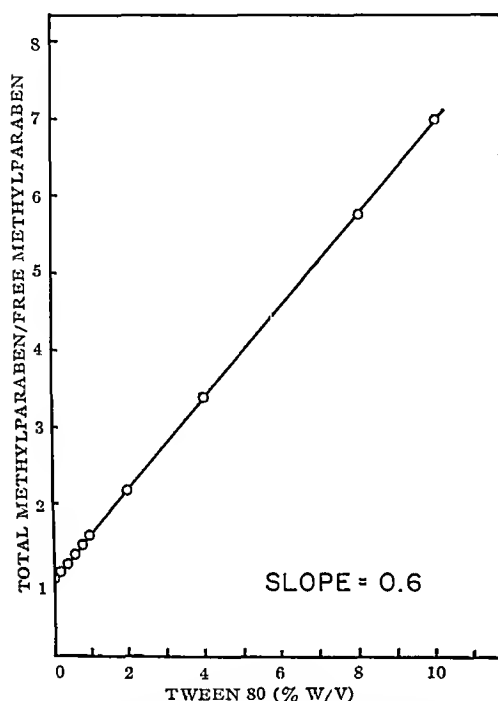


Fig 7—Ratio, r , of total methyl *p*-hydroxybenzoate to free methyl *p*-hydroxybenzoate obtained from the solubility study illustrated in Fig. 6.

water solubility of the paraben; and the concentration of bound paraben was obtained as the difference between the total paraben in solution and the free paraben concentration. Comparison of Figs. 3 and 7 indicates that the values obtained for the slope in the solubility and dialysis studies are in fairly good agreement. This suggests that the solubilization of the methyl *p*-hydroxybenzoate and the binding as determined in the dialysis studies can probably be attributed to the same mechanism. This agreement between the solubility study and the dialysis study also supports the validity of the assumption that any binding of Tween® by the nylon membrane can be neglected in the dialysis studies.

SUMMARY

1. The interaction of the methyl and propyl esters of *p*-hydroxybenzoic acid with Tween® 80 has been studied quantitatively by means of a dialysis method employing a semipermeable nylon membrane.

2. A relatively high degree of interaction has been observed, and the binding has been found to be a function of both the concentration of unbound *p*-hydroxybenzoate and the concentration of Tween® 80. At a concentration of five per cent Tween® 80 only 22 per cent of the total methyl *p*-hydroxybenzoate and 4.5 per cent of the total propyl *p*-hydroxybenzoate are present as unbound preservative.

3. A study of the solubility of methyl *p*-hydroxybenzoate in aqueous solutions of Tween® 80 indicated that the mechanism of the binding observed in the dialysis studies is probably the same mechanism which is responsible for the solubilization of the preservative by the Tween®.

4. The data obtained in this study facilitate calculation of the quantity of methyl or propyl *p*-hydroxybenzoate which must be added to a system containing a known concentration of Tween® 80 in order to have the desired concentration of unbound preservative.

REFERENCES

- (1) Bolle, A., and Mirimanoff, A., *J Pharm and Pharmacol*, **2**, 685(1950)
- (2) Bouchardy, M., and Mirimanoff, A., *Pharm Acta Helv*, **26**, 69(1951)
- (3) Bolle, A., *ibid*, **26**, 284(1951)
- (4) Lawrence, C. A., and Erlandson, A. L., *THIS JOURNAL*, **42**, 352(1953)
- (5) Okabayashi, T., *J Fermentation Technol (Japan)*, **31**, 292(1953), *Chem Abstr* **48**, 5276j(1954)
- (6) Aoki, M., Kamata, A., Yoshioka, I., and Matsuzaki, T., *J Pharm Soc Japan*, **76**, 939(1956)
- (7) deNavarre, M. G., and Bailey, H. E., *J Soc Cosmetic Chemists*, **7**, 427(1955)
- (8) deNavarre, M. G., *ibid*, **8**, 68(1957)
- (9) Barr, M., and Tice, L. F., *THIS JOURNAL*, **46**, 445(1957)
- (10) Eisman, P. C., Cooper, J., and Jaconia, D., *ibid*, **46**, 144(1957)
- (11) Sherwood, M. B., *J Dermatol*, **43**, 778(1942)
- (12) Quisno, R. A., Gibby, I. W., and Foter, M. J., *THIS JOURNAL*, **35**, 317(1946)
- (13) Allawala, N. A., and Riegelman, S., *ibid*, **42**, 267(1953)
- (14) Allawala, N. A., and Riegelman, S., *ibid*, **42**, 396(1953)
- (15) Higuchi, T., and Lach, J. L., *ibid*, **43**, 465(1954)
- (16) Guttman, D., and Higuchi, T., *ibid*, **45**, 659(1956)
- (17) Kostenbauder, H. B., and Higuchi, T., *ibid*, **45**, 518(1956)
- (18) Kostenbauder, H. B., and Higuchi, T., *ibid*, **45**, 810(1956)
- (19) Schonfeldt, N., *Nature*, **172**, 820(1953)
- (20) Schonfeldt, N., *J Am Oil Chemists' Soc*, **32**, 77(1955)
- (21) Werden, M. J. H., and Norton, L. B., *J Colloid Sci*, **5**, 606(1953)

The *In Vitro* Antibacterial Activity of Essential Oils and Oil Combinations*

By JASPER C. MARUZZELLA and PERCIVAL A. HENRY

The *in vitro* antibacterial activity of thirty-five volatile oils, five fixed oils, five infused oils, and ninety-five combinations of these oils was ascertained using the filter paper disk method. The oils and oil combinations were tested against five bacteria. Eucalyptus, cinnamon, and origanum red exhibited the greatest antibacterial activity while the volatile oils of cedar wood and myrrh as well as all of the fixed and infused oils were found to possess no activity. The Gram-positive bacteria were more susceptible to the volatile oils than the Gram-negative. Of the ninety-six combinations of oils only three were found to exhibit greater antibacterial activity as compared with each oil used separately. The three combinations of volatile oils which showed this enhancement of antibacterial activity were: eucalyptus-cinnamon-dwarf pine needle, eucalyptus-cinnamon-juniper berries, and eucalyptus-cinnamon-niaouli. When fixed or infused oils were added to volatile oils the antibacterial activity of the latter was markedly diminished.

THE MAJORITY of the testing for the antibacterial action of oils and their constituents has been performed with the well-known phenol coefficient method (1-11). Using this method it was found that emulsified mixtures of two or more oils increased the bactericidal efficiency as compared with each principle used separately (12). Recently the filter paper disk method has been employed to measure the antibacterial activity of oils (13, 14). The present investigation is concerned with the *in vitro* antibacterial activity of oils with special consideration to mixtures of two or more oils in order to ascertain whether antibacterial activity would be enhanced or diminished.

MATERIALS AND METHODS

The detection of the antibacterial activity of various volatile oils, fixed oils, and infused oils in addition to mixtures of two or more volatile oils, volatile and fixed oils, and volatile and infused oils was studied on growing cultures of five organisms. The organisms used were: *Salmonella typhosa*, *Micrococcus citreus*, *Proteus morgani*, *Bacillus brevis*, and *Micrococcus pyogenes var. albus*. Thirty-six hour cultures of these organisms were grown in nutrient broth (Difco). Stock cultures were maintained on fresh nutrient agar slants and subcultured every seven days.

The method used for determining the presence or absence of antibacterial activity was a slight modification of Vincent's (15) qualitative filter paper disk diffusion plate method. In this method small sterile disks (12.5 mm. diameter) of filter paper were thoroughly saturated with the oil or oil mixture and then placed on nutrient agar (Difco) plates which had been previously seeded with 2 cc. of a thirty-six hour nutrient broth culture of the organism. All dishes were conducted in triplicate with two or three disks per dish. The measurement of each zone of inhibition represents a mean value of a minimum of six

recordings. All dishes were incubated at 37° for twenty-four hours. The presence of a zone of inhibition of any size surrounding the paper disk indicated antibacterial activity. The zone of inhibition was measured to 0.5 mm. by means of a metric ruler and an illuminated Quebec bacteria colony counter.

As the oils arrived at the laboratory they were placed into sterile bottles and tested for sterility by streaking on nutrient agar slants and incubated at 37° for one to three days. All of the oils used were found to be free of bacteria. Oil combinations were prepared by adding the appropriate quantity to one another and mixing thoroughly in a sterile bottle.

RESULTS AND DISCUSSION

From Table I it may be observed that of the thirty-five volatile oils tested, thirty-three were found to have antibacterial activity on at least one of the five organisms employed. Volatile oils of cedar wood and myrrh were found to have no activity. The five fixed oils, castor, cod liver, vitamin K₁, olive, and white mineral, and the five infused oils, asafetida, burdock, henbane, lobelia, and mullein, were found to possess no antibacterial activity. The effect of the volatile oils against each organism was found to be of the following order (figures indicate the sum of the zones of inhibition caused by the oils): *M. citreus*, 174 mm.; *B. brevis*, 168 mm.; *Micrococcus pyogenes var. albus*, 102 mm.; *P. morgani*, 63 mm.; and *S. typhosa*, 48 mm. Therefore the volatile oils are more effective against Gram-positive bacteria than against Gram-negative. This is in agreement with the work of Turkheim (13) and Maruzzella and Lichtenstein (14) who likewise found volatile oils to be more effective against Gram-positive bacteria. Furthermore the latter investigators also obtained negative results with fixed and infused oils on bacteria. It may also be observed from Table I that the volatile oils showing the greatest sum total of zones of inhibition were: eucalyptus, cinnamon, and origanum red.

Table II shows the results obtained on bacteria with 47 combinations of two volatile oils (1:1), 15 combinations of three volatile oils (1:1:1), 28 combinations of volatile and fixed oils (1:1), and five combinations of volatile and infused oils (1:1). With

* Received June 1, 1957, from Long Island University, Biology Department, Brooklyn, N. Y.

TABLE I —INHIBITORY ACTIVITY OF OILS

Volatile Oils	Zone of Inhibition, mm ^a					Sum ^b
	S- ty- phosa	M- ci- treus	P- mor- gani	B- bre- vis	M- al- bus	
Amber, Rect	0 ^c	2	0	2	2	6
Anise	0	3	0	4	2	9
Bay	0	7	3	3	3	16
Bergamot	4	7	0	6	3	20
Cajuput	0	10	0	11	5	26
Caraway	0	10	0	5	3	18
Cedar Wood	0	0	0	0	0	0
Chenopodium	0	5	3	3	3	14
Cinnamon	0	11	8	12	10	41
Clove	0	3	0	3	2	8
Cubeb	0	0	0	2	0	2
Dwarf Pine						
Needle	3	3	5	3	3	17
Eucalyptus	5	10	10	12	8	45
Geranium						
Algerian	2	12	2	5	2	23
Hemlock	1	0	2	0	0	3
Juniper Berries,						
Rect Dutch	5	2	9	2	3	21
Juniper Tar	0	10	0	2	3	15
Lemon	0	0	0	13	0	13
Myrrh	0	0	0	0	0	0
Neroli	5	3	4	10	3	25
Niaouli	0	10	6	10	3	29
Organum Red	0	15	0	15	10	40
Peppermint	5	0	0	5	2	12
Pettigrain						
Paraguay	3	0	3	4	2	12
Pimento	0	10	0	4	3	17
Pinus Sylvestris	8	5	0	6	5	24
Rectified Tar	0	10	0	0	6	16
Rosemary	5	7	0	5	6	23
Sandalwood	0	3	0	2	2	7
Sassafras	0	3	0	3	2	8
Spike Lavender	0	5	0	5	3	13
Sweet Birch	0	0	0	2	0	2
Sweet Orange	0	0	0	2	0	2
Turpentine	2	3	8	4	3	20
Wintergreen	0	5	0	3	0	8

^a Measurement from disk edge to zone edge
^b Sum of zones of inhibition for each oil
^c Zone of inhibition absent

TABLE II (Continued)

Oil Combinations	Zone of Inhibition, mm ^a					Sum ^b
	S- ty- phosa	M- ci- treus	P- mor- gani	B- bre- vis	M- al- bus	
Fucalypthus						
Cinnamon	0	12	0	15	15	42
Fucalypthus						
Clove	0	5	0	5	5	15
Eucalyptus						
Cubeb	5	5	4	5	6	25
Eucalyptus-						
Dwarf Pine						
Needle	8	10	0	8	6	32
Eucalyptus						
Juniper Berries	8	9	0	10	5	32
Eucalyptus						
Myrrh	3	5	0	5	3	16
Eucalyptus-						
Niaouli	0	8	0	12	5	25
Eucalyptus Pinus						
Sylvestris	7	10	0	15	3	35
Eucalyptus-						
Rosemary	9	8	0	10	9	36
Fucalypthus-						
Sandalwood	3	5	5	4	5	22
Eucalyptus-						
Sweet Orange	7	5	0	6	4	22
Eucalyptus-						
Wintergreen	0	5	0	5	5	15
Juniper Berries-						
Cedar Wood	2	0	3	2	2	9
Juniper Berries-						
Dwarf Pine						
Needle	2	2	7	2	2	15
Niaouli Clove	0	5	0	4	3	12
Niaouli-Cubeb	0	2	3	3	1	9
Niaouli-Pinus						
Sylvestris	0	8	0	12	7	27
Niaouli-Rectified						
Tar	0	6	0	6	4	16
Niaouli-Sweet						
Birch	0	4	0	4	3	11
Niaouli-Winter-						
green	0	3	0	4	3	10
Peppermint-						
Pimento	0	4	0	3	3	10
Turpentine Clove						
Turpentine-	3	5	0	1	4	16
Hemlock						
Turpentine-	0	0	4	2	3	9
Juniper Berries						
Turpentine-	3	2	9	2	3	19
Organum Red						
Turpentine-	0	10	0	0	8	18
Wintergreen						
Turpentine-	0	5	0	4	3	12
Volatile (1 1 1)						
Cedar Wood-						
Cubeb-Bay	0	3	2	3	2	10
Eucalyptus-						
Chenopodium-						
Anise	0	5	0	3	4	12
Eucalyptus-						
Chenopodium-						
Cedar Wood	4	6	0	4	5	19
Eucalyptus						
Chenopodium-						
Hemlock	5	6	0	5	4	20
Eucalyptus-						
Chenopodium-						
Peppermint	3	5	0	4	3	15
Eucalyptus						
Cinnamon-						
Clove	0	4	3	4	3	14
Eucalyptus						
Cinnamon-						
Dwarf Pine						
Needle	8	11	0	15	18	51
Eucalyptus-						
Cinnamon-						
Juniper Berries	10	12	0	17	19	58
Eucalyptus-						
Cinnamon-						
Niaouli	7	11	0	15	15	51
Fucalypthus-						
Cinnamon-						
Rosemary	0	5	3	6	4	18
Sassafras-						
Organum Red-						
Chenopodium	0	5	3	3	3	14
Turpentine-						
Geranium						
Algerian						
Rosemary	0	5	3	6	5	19
Turpentine-						
Neroli						
Geranium						
Algerian	0	2	2	3	5	12

TABLE II —INHIBITORY ACTIVITY OF COMBINATIONS OF OILS

Oil Combinations	Zone of Inhibition, mm ^a					Sum ^b
	<i>S</i> <i>ty-</i> <i>phosa</i>	<i>M</i> <i>ci</i> <i>treus</i>	<i>P</i> <i>mor-</i> <i>gani</i>	<i>B</i> <i>bre-</i> <i>vis</i>	<i>M</i> <i>al-</i> <i>bus</i>	
	Volatile (1 1)					
Anise Clove	0 ^c	3	0	4	2	9
Anise Hemlock	0	3	0	4	2	9
Chenopodium-						
Cedar Wood	0	10	0	11	7	31
Cinnamon Clove	0	10	0	10	10	30
Cinnamon Cubeb	0	10	0	13	10	33
Cinnamon-Dwarf						
Pine Needle	0	15	0	15	5	35
Cinnamon-Juniper Berries	0	10	0	15	5	30
Cinnamon-						
Niaouli	0	10	0	11	10	31
Cinnamon-Pinus Sylvestris	0	10	0	12	3	25
Cinnamon-Sandalwood	0	10	0	12	5	27
Cinnamon Sweet Birch	0	7	0	10	3	20
Cinnamon-Turpentine	0	10	0	15	14	39
Cubeb Cedar Wood	0	0	0	1	0	1
Cubeb-Dwarf Pine Needle	3	2	4	3	2	14
Cubeb-Juniper Berries	3	2	5	2	3	15
Cubeb Pinus Sylvestris	2	3	0	2	1	8
Dwarf Pine Needle Cedar Wood	3	2	4	2	2	13
Eucalyptus Anise	0	5	0	5	5	15
Eucalyptus Cajuput	0	4	0	12	5	21
Eucalyptus-Cedar Wood	3	5	0	5	5	18
Eucalyptus-Chenopodium	0	9	0	8	6	23

TABLE II (Continued)

Oil Combinations	Zone of Inhibition, mm ^a					Sum ^b
	<i>S. typhosa</i>	<i>M. citreus</i>	<i>P. mor- gani</i>	<i>B. bre- vis</i>	<i>M. al- bus</i>	
Wintergreen-Sweet Birch-Lemon	0	2	0	2	0	4
Wintergreen-Sweet Orange-Sassafras	0	4	0	3	0	7
Volatile and Fixed (1 1)						
Castor-Cedar Wood	0	0	0	0	0	0
Castor-Eucalyptus	0	5	0	1	5	14
Castor-Origanum Red	0	2	0	3	0	5
Castor-Turpentine	0	0	0	1	0	1
Cod Liver-Cedar Wood	0	0	0	0	0	0
Cod Liver-Eucalyptus	0	5	0	4	7	16
Cod Liver-Origanum Red	0	3	0	4	3	10
Cod Liver-Turpentine	0	0	0	2	2	4
Olive-Amher, Rect	0	0	0	2	0	2
Olive-Bay	0	2	0	3	3	8
Olive-Bergamot	0	1	0	2	2	5
Olive-Cajuput	0	5	0	4	5	14
Olive-Caraway	0	0	0	2	0	2
Olive Cedar Wood	0	0	0	0	0	0
Olive-Chenopodium	0	2	0	3	0	5
Olive-Clove	0	3	0	2	2	7
Olive-Eucalyptus	0	5	0	4	8	17
Olive-Juniper Tar	0	2	0	2	2	6
Olive Origanum Red	0	4	0	5	2	11
Olive-Peppermint	0	0	2	2	0	4
Olive-Sassafras	0	0	0	1	0	1
Olive-Turpentine	0	2	4	1	0	7
Olive-Wintergreen	0	0	0	0	0	0
Vitamin K ₁ -Eucalyptus	0	7	10	4	6	27
White Mineral-Cedar Wood	0	0	0	0	0	0
White Mineral-Eucalyptus	0	7	0	5	7	19
White Mineral-Origanum Red	0	7	0	8	0	15
White Mineral-Turpentine	0	2	6	2	3	13
Volatile and Infused (1 1)						
Asafetida-Eucalyptus	0	3	3	3	5	14
Burdock-Eucalyptus	0	3	5	2	5	15
Henbane-Eucalyptus	0	6	5	5	5	21
1 obelia-Eucalyptus	0	5	7	5	5	22
Mullein-Eucalyptus	0	6	3	3	5	17

^a Measurement from disk edge to zone edge^b Sum of zones of inhibition for each oil.^c Zone of inhibition absent

the combinations of two volatile oils none were found to enhance antibacterial activity. Two combinations, anise-clove and anise-hemlock, were found to have the same effect as anise used alone. The

remaining 45 mixtures of two volatile oils showed a decrease in antibacterial activity as compared to each oil used separately. With the combinations of three volatile oils the following mixtures were found to exhibit greater antibacterial activity as compared to each oil used separately (compare Tables I and II): eucalyptus-cinnamon-dwarf pine needle, eucalyptus-cinnamon-juniper berries, and eucalyptus-cinnamon-niaouli. The remaining combinations of three volatile oils were found to produce a decrease in antibacterial activity. All mixtures of volatile with fixed oils and volatile with infused oils resulted in a marked decrease in the antibacterial activity of the volatile oil.

The mechanism of action of the antibacterial activity of volatile oils is not known but some clues are given by the work of Gal'perin and Dunaeva (16, 17). These investigators found that many volatile oils produced arresting effects on dehydrogenases of paramacia and helminths. This may also be true of bacteria.

The reason why most oil combinations result in a decrease in antibacterial activity is likewise unknown. Perhaps a loss of power of diffusion would explain this phenomenon. Indeed this would seem to be true for mixtures of volatile oils with fixed and infused oils. Furthermore it would not be unreasonable to assume that fixed and infused oils might coat the active constituents of the volatile oils rendering them inactive to the bacterial enzymes. In the work of Miller (12) mixtures of volatile oils were found to enhance bactericidal power by killing the organisms in a shorter period of time. With the method used in this investigation enhancement of antibacterial power was indicated by an increase in the size of the zone of inhibition produced at a fixed period of time. With these different methods it is possible to obtain divergent results.

REFERENCES

- (1) Martindale, W. H., *Perfumery Essent. Oil Record*, 2, 10(1911)
- (2) Martindale, W. H., *Pharm J*, 85, 1911.
- (3) Baker, R. T., and Smith, H. G., *Med J. Australia*, 2, 101(1919)
- (4) Wood, H. C., *This Journal*, 9, 878(1920).
- (5) Penfold, A. R., and Grant, R. J., *J. Proc. Roy. Soc., N. S. Wales*, 60, 167(1926).
- (6) Rideal, S., Rideal, E. K., and Seiver, A., *Perfumery Essent Oil Record*, 19, 285(1928).
- (7) Rideal, A., Seiver, A., and Richardson, N. E. G., *ibid*, 21, 341(1930).
- (8) De, P. K., and Subrahmanyam, V., *Indian J. Med. Research*, 17, 1153(1930)
- (9) Naves, Y. R., *Parfums France*, 13, 273(1935).
- (10) Piacentini, G., *Ann igiene*, 58, 1(1948)
- (11) Bose, S. M., Bhima Rao, C. N., and Subrahmanyam, V., *J. Sci. Ind. Research*, 8B, 157(1949).
- (12) Miller, R. R., *Am J. Pharm.*, 103, 324(1931)
- (13) Turckheim, H. J., *J. Dental Research*, 28, 677(1949)
- (14) Maruzzella, J. C., and Lichtenstein, M. B., *This Journal*, 45, 378(1950)
- (15) Vincent, J. G., and Vincent, H. W., *Proc. Soc. Exptl Biol Med*, 55, 162(1944)
- (16) Gal'perin, I. M. M., and Dunaeva, V. F., *Ukrain. Biokhim. Zhur*, 26, 451(1954)
- (17) Gal'perin, I. M. M., and Dunaeva, V. F., *ibid*, 27, 197(1955).

Comparative Dissolution Rates of Weak Acids and Their Sodium Salts*

By EINO NELSON

The dissolution rates of several weak acids and their sodium salts were studied in mediums representing gastrointestinal fluids with respect to pH. Marked differences existed between dissolution rates of acids and their sodium salts; the sodium salts always dissolved much more rapidly than the free acid. The experimental method was based on solution by free convection and an expression was derived to express dissolution rate from a circular plate suspended vertically in dissolution medium as a function of the variables involved. The validity of the expression was shown for sodium chloride dissolving in water. The significance of the results in acid-salt rate studies to oral administration of drugs was discussed.

IT WAS SHOWN in some recent work that marked differences existed in the rate with which several amine and alkanolamine salts of the weak acid theophylline dissolved (1). It was suggested that these differences were related to theophylline blood levels after oral administration of the salts in clinical studies. It was also shown that all salts dissolved more rapidly than the free acid in a pH range representing those likely to be encountered by drug after oral administration. In this pH range, the rates of solution of both the free acid and the salts were relatively independent of medium.

The differences in rate observed between the salts and between any salt and the free acid were attributed to the properties of the diffusion layer which surrounds a dissolving solid. The pH of the diffusion layer surrounding a salt of a weak acid, if the salt is a highly water-soluble compound will be relatively independent of dissolution medium pH and this property will exert a strong influence on rate of solution. Generally speaking, rate will increase with diffusion layer pH since concentration increases with this quantity but other factors are also involved.

In view of the above, a marked difference should exist in the rate with which the sodium and potassium salts of weak acids dissolve in comparison to the free acids. The work now reported was conducted to determine the comparative solution rates for several weak acids and their sodium salts in mediums representing gastrointestinal fluids with respect to pH. Another purpose of the work was to develop a technique for studying dissolution rate without requiring an assay method for the dissolving specie.

EXPERIMENTAL

Theory of the Method.—Dissolution rate was studied by a method in which free convection, diffusion coefficient and concentration in the diffusion layer were the rate determining factors. An expression to relate average rate per unit area from a two dimensional rectangular surface, suspended vertically in dissolution medium, with the several parameters involved in the process has been previously

$$R'_m = 0.726 DC_s \left(\frac{g}{HD\nu} \frac{\Delta\rho}{\rho_0} \right)^{1/4} \quad (\text{Eq. 1})$$

described (2). This expression, describes mean rate per unit area in terms of diffusion coefficient D , concentration in the diffusion layer C_s , height of the rectangular plate H , viscosity of the diffusion layer ν , difference in density between the diffusion layer and the medium $\Delta\rho$, gravitational constant g and density of the medium ρ_0 (all terms in cgs units). The correctness of this expression to describe rate of dissolution of thin plates of sodium chloride in water was established (2).

The test specimens used in the present work were thin compressed circular pellets of several weak acids and their sodium salts mounted in a manner such as to allow vertical suspension in dissolution medium. The dissolution rate observed from a circular surface should also be a mean rate since rate varies with height of infinitesimally thin vertical sections across the surface. This mean rate should be expressible by the mean value of equation 1 after relating the quantity H to an appropriate variable. The quantity H may be expressed as a function of r and θ , where r is the radius of the circular surface; the quantity θ is the angle between a horizontal line through the center of the surface and any given radius forming the third side of a right triangle bounded by these two lines and a vertical line whose length is one-half the length of a corresponding infinitesimally thin vertical section. Expressing H in terms of r and θ the mean value of equation 1 will be given by evaluation of the following expression:

$$R_m = \int_{\pi/180}^{\pi/2} \frac{0.462DC_s}{\sin^{1/4}\theta} \left(\frac{g}{2rD\nu} \frac{\Delta\rho}{\rho_0} \right)^{1/4} d\theta \quad (\text{Eq. 2})$$

* Received June 6, 1957 from the School of Pharmacy, University of California Medical Center, San Francisco 22, California

In equation 2 the lower limit must be taken at some small angle since the integral has no limit at $\theta = 0$. This is an academic consideration only and equation 2 may be integrated graphically to give

$$R_m = 0.884DC_s \left(\frac{g}{2rDv} \frac{\Delta p}{\rho_a} \right)^{1/4} \quad (\text{Eq. 3})$$

It is seen from equation 3 that dissolution rate observed from a circular surface will be constant at constant temperature and surface area. It is implicitly understood that concentration in the medium is always small with respect to the concentration of the diffusion layer.

Preparation of Test Samples.—The test pellets were prepared from powdered samples of the weak acids and their sodium salts by compressing in a $1/2$ -in. die with flat-faced punches at 50,000 psi. The compressions were made in a Carver press modified to allow compression using standard tablet machine punches and dies. The die wall and punch faces were lubricated with a slurry of magnesium stearate in alcohol prior to compressions. All compounds were of USP, NF or better quality and used without further purification. The compounds used are listed in Table I. Test pellets of sodium chloride used to check the validity of equation 3 were prepared from -200 mesh analytical reagent grade chemical by compression in vacuum by a technique previously described (1). These pellets were 1.3 cm. in diameter. In the case of weak acids and their sodium salts it was sometimes necessary to precompress the powders before intact pellets could be formed.

The pellets prepared as described above were mounted flat on ceresin wax coated aluminum strips 2 cm. wide by 4 cm. long.

The apparent density of all pellets was determined by micrometer measurements and weighings.

Test Procedure for Sodium Salts.—The mounted pellets were suspended by means of the nylon thread to the suspension hook of a Sartorius Selecta balance in such a manner as to allow immersion of the entire mounting plate with specimen in 175 ml. dissolution medium positioned over the balance pan. The weight loss was followed directly on the illuminated 0 to 100 mg. scale. No weight changes were recorded until all rotational movement of the test pellet and its mounting had ceased. After equilibrium was established, the times required for apparent losses of 4 or 5 mg. were noted with a stop watch. Readings were recorded only during the dissolution of the first 30 to 40 mg. since a wax wall was left around the undissolved part of the pellets. The presence of this wall was considered to cause a change in the flow pattern across the surface of the specimens. The apparent losses of weight multiplied by the ratios of the densities of the pellets to the difference between the densities of the pellets and mediums gave the amounts dissolved.

The calculations involved in expressing the observed weight losses to the rate units of Table I are illustrated by the following: On the average, an apparent weight loss of 0.0972 mg. per sec. was observed for sodium benzoate dissolving in 0.1 *M* borate buffer. The density of 0.1 *M* borate buffer at 25° was 1.018 and the apparent density of the sodium benzoate pellets was 1.426. Multiplying the apparent loss by the ratio calculated from these densities as described gave an actual loss of 0.3396 mg. per sec. This quantity divided by the area of the pellet, 1.275 sq. cm. gave a rate per unit area of 0.2661 mg./sec.-cm.² This quantity multiplied by 6000 to convert to hundreds of minutes gave the quantity listed in Table I.

TABLE I.—DISSOLUTION RATE OF WEAK ACIDS AND THEIR SODIUM SALTS IN VARIOUS BUFFERS^{a,b}

Compound	0.1 <i>N</i> HCl pH = 1.5	0.1 <i>M</i> Phosphate pH = 6.83	0.1 <i>M</i> Borate pH = 9.00	Solubility of free acid Gm/l., 25°	pKa, 25°
Benzoic acid	2.1	14	28	2.9°	4.2°
Sodium salt	980	1770	1600		
Phenobarbital	0.24	1.2	22	1.0°	7.4 ^f
Sodium salt	~200	820	1430		
Salicylic acid	1.7	27	53	2.2°	2.98°
Sodium salt	1870	2500	2420		
Succinic acid	2100	310	310	~77°	4.18 ^{d,e}
Sodium salt	6000	3360	2920		
Sulfathiazole	<0.1	~0.50	8.5	0.6°	7.26°
Sodium salt	550	810	1300		

^a Rate = mg./100 minutes-cm.² ^b Solution process by free convection. ^c From reference 3. ^d From Reference 4. ^e First hydrogen. ^f From Reference 5. ^g From Reference 6.

Each strip had a small hole punched near the top edge which allowed suspension by means of nylon sewing thread. Pellet mounting was by means of the coating wax and additional molten wax was used to cover the peripheral surface of the pellets thus leaving only one circular face exposed. Before use in tests, the pellets were carefully scraped with a razor blade to remove the excess wax from their edges and lubricant from their faces.

The surface of the pellets were observed during tests to detect the formation of air bubbles since their presence would indicate permeation of solvent into the pellets' interstices.

The test mediums were brought to 25° for tests and since the tests could be made rapidly the change in temperature during tests did not exceed 0.5°. The dissolution rate of sodium chloride was determined at 16° in water.

Procedure for Acids.—With the exception of succinic acid, whose dissolution rate was studied by the same method as the sodium salts, all acids required immersion in dissolution medium maintained at 25° by means of a constant temperature bath. The mounted samples were weighed in air after equilibration with the atmosphere for several days. They were then immersed in dissolution medium for periods up to twenty-four hours, removed, rinsed with distilled water, and left to dry again in the atmosphere. Essentially constant weight was reached in twenty-four hours drying by this method. This method was necessary since it was not practical to continuously observe the pellets for permeation of solvent into their interstices. The loss of weight was recorded and expressed in terms of the appropriate rate units.

RESULTS AND DISCUSSION

Test of Equation 3.—The calculated value for the dissolution rate of sodium chloride at 16°, using the values for the parameters used to check the validity of equation 1 previously (2), was 30.4 mg./min.-cm.² Three determinations with compressed disks of sodium chloride gave values of 29.8, 30.6, and 30.1 mg./min.-cm.² respectively.

Dissolution Rate of the Acids and Salts.—The rate found for the materials studied are listed in Table I along with other information of interest. Determinations were reproducible to within 10% of the mean of three or four determinations. Observation of the pellets during and after tests gave no indication that disintegration occurred.

It is strikingly apparent from examination of Table I that large differences existed between the solution rates of all acids and their sodium salts. Further, the difference existed when the free acid was a relatively water-soluble compound as was the case with succinic acid. The increase in rate of dissolution of the poorly soluble free acids with increasing pH of dissolution medium was expected when their dissociation constants were large enough to allow for the formation of significant quantities of anion in the diffusion layers—particularly in the borate buffer.

A decrease in rate of solution of the salts could be expected with increasing buffer capacity of the dissolution medium. Increasing buffer capacity of the medium would tend to overcome the buffer capacity of the salt in the diffusion layer. Conversely, an increase in rate of solution of the free acids could be expected with an increase in buffer capacity of alkaline dissolution mediums as was the case with theophylline (1).

The absolute difference in rates between acids and their salts was somewhat dependent on the solution process. This is due to the fact that flow rate across the face of the pellets varied in accordance with the density difference term in equation 3. Hence, the differences in rates observed here were greater than those which would be observed were solution taking place under conditions where the Noyes-Whitney law described the solution rate process.

When the salts of poorly soluble weak acids dissolved in mediums that had an insignificant capacity for the anion and small capacity for the free acid, c g, 0.1 N HCl, precipitation of free acid neces-

sarily occurred after the diffusion layer mixed with solvent. The significance of this occurrence when considering the oral administration of therapeutically active weak acids as sodium salts is in the increase in surface area of administered material obtained in the stomach. This increase in surface would increase rate of solution and consequently cause an increase in absorption rate. This consideration was no doubt a factor in explaining the higher and the more rapidly reached maximum blood levels obtained from the administration sodium aspirin in solution as compared to aspirin in tablets (7).

Comments on the Significance of Solution Rate in Absorption of Drugs.—It is appropriate to mention that an understanding and knowledge of the rate of solution of drugs administered in solid form is of importance. In general, availability for absorption is completely dependent on drug being in solution at absorption sites and the rate of the solution step may markedly influence the rate of build-up and maximum level of drug reached in the fluids of distribution.

Absorption of drugs is commonly related to their solubility by many investigators in fields where the subject is pertinent. Relating absorption to solubility is not correct. Solubility refers to the maximum amount of material that can be dissolved in a given volume of solvent at constant temperature in any time long enough to allow attainment of saturation. The absorption of drugs is a dynamic process and ultimate solubility of a drug in fluid at absorption sites is of limited consequence since absorption prevents the attainment of saturated solutions. Solubility of a drug and absorption are related only to the extent that solubility affects solution rate. Solution rate influences absorption rate since it is a preceding process. In addition to solubility, solution rate is dependent on surface, diffusion coefficient and other properties of the dissolving species and the solution process. The condition that exists in the diffusion layer of the salts of weak acids as shown in the work of this paper is another consideration. It should be clear, then, that absorption is properly related to solution rate, not solubility.

SUMMARY

The dissolution rate of the sodium salts of weak acids is much more rapid than the free acids. This is true in mediums possessing hydrogen ion concentrations in the range of those encountered by drugs administered orally. The significance of solution rate in the administration of drugs was discussed.

REFERENCES

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- (2) Wagner, C. *J. Phys. Chem.*, **53**, 1030 (1949).
- (3) "Merck Index", 6th ed., Merck and Company, Rahway, N. J., 1952.
- (4) Hodgman, C. D., "Handbook of Chemistry and Physics," 30th Edition, Chemical Rubber Publishing Co., Cleveland, Ohio, 1947, p. 1425.
- (5) Kral, M. E. *J. Phys. Chem.*, **44**, 449 (1940).
- (6) Higuchi, T., Gupta, M., and Busse, L. W., *THIS JOURNAL*, **42**, 157 (1953).
- (7) Carlo, P. E., Cambozos, N. M., Feeney, G. C., and Smith, P. K., *ibid.*, **44**, 396 (1955).

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- (6) Higuchi, T., Gupta, M., and Busse, L. W., *THIS JOURNAL*, 42, 157 (1953).
- (7) Carlo, P. E., Cambosio, N. M., Feeney, G. C., and Smith, P. K., *ibid.*, 44, 396 (1955).

Dissolution Rate of Mixtures of Weak Acids and Tribasic Sodium Phosphate*

By EINO NELSON

The dissolution rates of mixtures of several weak acids and trisodium phosphate were determined in stirred mediums and by a process whereby dissolution took place by free convection. The test samples were pelleted mixes of weak acid and trisodium phosphate in a range of mole fraction ratios. The admixture of tribasic sodium phosphate increased the rate of solution of all acids studied and a characteristic relationship was found between rate and fraction of acid in mixture. In all cases, a maximum occurred in plots of rate vs fraction and its location with respect to amount of acid in a mix appeared to be related to the strength of the acid. The maximum rates obtained for the mixes were lower than those obtained from salts of the acids.

THE KINETICS of the solution process have been thoroughly investigated for single substances since the first studies of Noyes and Whitney (1). Apparently no studies have been made when the substance dissolving consists of two or more components. The latter studies are of pharmaceutical interest since many such systems are commonly encountered in dosage forms e.g., active ingredient plus binder and other materials in tablets or tablet granules and salts of weak acids mixed with buffer salts such as benzylpenicillin sodium with sodium citrate.

Another two component system of pharmaceutical convenience would be one composed of weak acid plus a strongly basic reacting salt. A rational use of the mixtures would lie in providing an environment near the dissolving mixtures in which the weak acids would be more soluble than the medium in which solution was taking place. In most cases, this condition would exist in or close by the diffusion or interfacial layer between the dissolving solid and medium. This same effect may be obtained in the case of poorly soluble weak acids by use of salts of the acids made with highly water soluble bases as has been previously shown (2), but some factors may make use of the salts themselves undesirable.

The work now reported was performed to determine the degree of enhancement in dissolution rate of poorly soluble weak acids obtainable by making homogeneous mixtures of these substances and tribasic sodium phosphate. It was also of interest to compare these rates to those obtained when salts of the acids were studied.

EXPERIMENTAL

Materials and Equipment.—The dissolution assembly and equipment for making 13 cm test pellets has been previously described (2). The

tests were made using the weak acids, theophylline, theobromine, and phenobarbital. These substances were recrystallized twice from boiling water and dried at 105°. Benzoic acid which was also used was recrystallized twice from boiling water and dried under partial vacuum over sodium hydroxide pellets at room temperature. The melting points of all compounds were in accord with literature values. The tribasic sodium phosphate used was the dodecahydrate of analytical reagent grade. The mixtures of acid with tribasic sodium phosphate were made of -200 mesh particles on a mole fraction basis.

Dissolution Rate Determinations.—Theophylline, theobromine, and benzoic acid-sodium phosphate mixtures were studied by the method previously used for theophylline salts in which solution took place from a constant surface in stirred medium (2). Phenobarbital-sodium phosphate mixture rates were determined by a method in which free convection, diffusion constant, and concentration in the diffusion layer were the rate determining factors (3). A few additional tests of the first mentioned mixtures were also made by this method. The rates per unit area or fluxes were calculated from the slopes of concentration *versus* time curves and expressed as $Gm/sec-cm^2$ for tests from stirred medium. Fluxes from determinations by free convection were available directly after correcting apparent rate by multiplying by the product of the ratio of the density of the pellet to the difference between the density of the pellet and medium and weight fraction acid in mixture. All determinations were made in water at 25° unless otherwise noted.

Analytical.—The concentration of acids in dissolution medium were determined spectrophotometrically. Theophylline was determined at 270 $m\mu$ (4) and theobromine at 273 $m\mu$ (5) after adjusting the solutions to 0.1 *N* with respect to HCl. Benzoic acid was also determined in 0.1 *N* HCl after establishing that the absorbance of its solutions at an absorption maximum at 230 $m\mu$ were linear to densities of at least 0.8. Its calculated molar extinction was 11,100 which was lower than a previously reported value of 11,600 (6). The value determined here was probably the more correct of the two, since it was obtained with calorific grade benzoic acid.

Pellet Density Determination.—The density of pellets was determined by measurements of thickness and diameter and weighing. The surface of

* Received August 5, 1957 from the School of Pharmacy, University of California Medical Center, San Francisco 22.

each pellet consisting of acid in the various mixes was calculated by the following expression:

$$F = \frac{\frac{w_a}{d_a}}{\frac{w_a}{d_a} + \frac{w_p}{d_p}} \quad (\text{Eq. 1})$$

In Eq. 1, F was the fraction of surface that consisted of acid in a compressed pellet, w_a was the weight of acid in the mixture, w_p the weight of tribasic sodium phosphate in the mixture and d_a and d_p , respectively, the apparent densities of theophylline and tribasic sodium phosphate after compression at the same pressure as the pellet mixes.

RESULTS AND DISCUSSION

Rate Determinations.—The concentration of drug in dissolution medium was found to increase linearly with time for all materials studied by the stirring process indicating that the reduced form of the Noyes-Whitney solution rate equation,

$$\frac{d_a}{dt} = KSC_s \quad (\text{Eq. 2})$$

described the process from any given two-component system. Equation 2 describes rate of solution taking place from a constant surface into a medium whose concentration is always low with respect to dissolving solid. In Eq. 2, a is the amount of material in solution, t is time, K is an apparent rate constant incorporating among other things the rate constants for both dissolving species, S is surface, and C_s is concentration of the compound of interest in the diffusion layer. The linearity of the relationship between concentration and time is shown in Fig. 1 for several compositions studied.

The fluxes calculated from concentration *versus* time plots for theophylline, theobromine and benzoic acid are plotted as a function of mole fraction of each acid in the mixtures in Fig. 2. Data for phenobarbital are also included. It was not necessary to calculate flux for this compound from concentration *versus* time plots since the experimental determination gave a rate directly convertible to the ordinate term shown as previously discussed.

The curves of Fig. 2 indicate that a complex relationship existed between flux and fraction of drug in a mixture. Further, a marked enhancement in rate was obtained in all cases when compared to the free acid. In Fig. 3 the observed flux for theophylline has been corrected to unit area theophylline and plotted *versus* fraction in mixture. In the region of linearity on this plot the rate-determining factor was the fraction of a pellet's surface that consisted of theophylline. Similar plots for the other materials studied could only be fitted by straight lines in more limited regions. The observed flux for all acids could be expected to be influenced by the hydrogen ion concentration existing in the diffusion layer. It was not practical to measure this quantity.

Examination of pellets after tests in stirred medium did not show surface roughness that would indicate that solution took place by a process other than near simultaneous release of both components. The composition consisting of $2/3$ mole phenobarbital with $1/3$ mole tribasic sodium phosphate

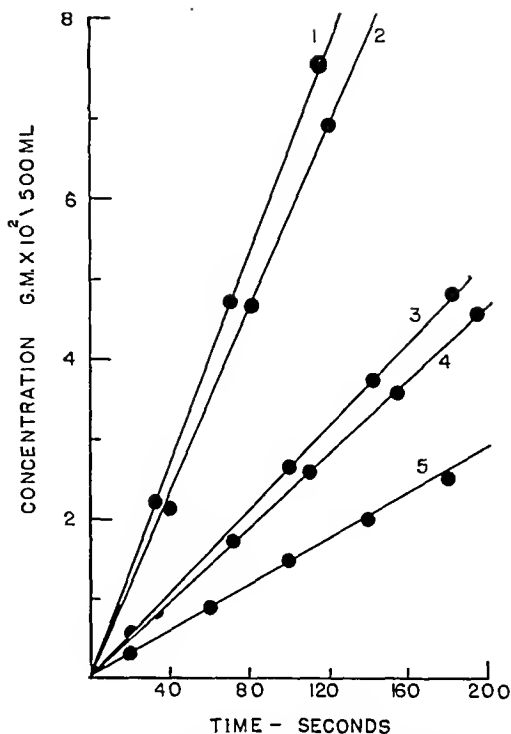


Fig. 1.—Illustrating depression in solution rate when 0.1 N HCl instead of water was used for dissolution medium with aminophylline and a theobromine composition consisting of $1/3$ mole theobromine with $2/3$ mole tribasic sodium phosphate. Curve 1—aminophylline in water, Curve 2—aminophylline in 0.1 N HCl, Curve 3—theophylline $2/3$ mole plus tribasic sodium phosphate $1/3$ mole in water, Curve 4—theobromine $1/3$ mole plus tribasic sodium phosphate $2/3$ mole in water, and Curve 5—the composition in 4 in 0.1 N HCl. Curves 3, 4, and 5 also illustrate the linear release with time of weak acids from these two component systems.

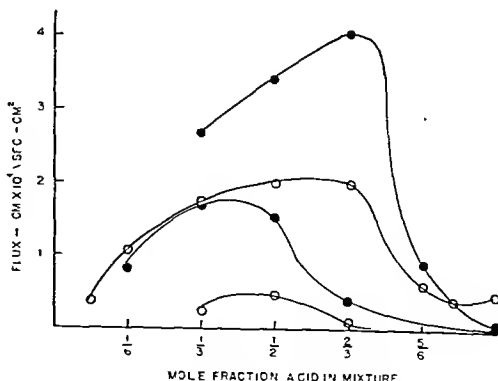


Fig. 2.—Illustrating the relationship between rate and fraction of acid for mixtures of weak acids and tribasic sodium phosphate. Dissolution in water at 25°. Curves from top to bottom are, respectively, for benzoic acid, theophylline, theobromine, and phenobarbital.

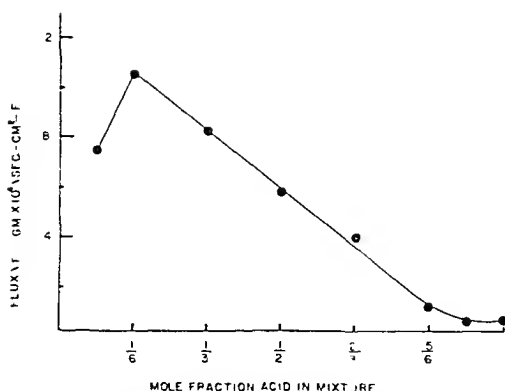


Fig. 3.—Illustrating region of dependency of solution rate of theophylline mixed with tribasic sodium phosphate on fraction of area presented to solvent by theophylline. F is the calculated fraction of a test pellet's surface that consists of theophylline.

studied by the free convection process was pitted after tests indicating simultaneous release did not take place. The composition consisting of $2/3$ mole benzoic acid and $1/3$ mole of the phosphate salt was carefully observed during dissolution by free convection. Solution took place in $0.1\ N$ HCl, in which medium only minute amounts of benzoic acid could dissolve. Precipitate formed only on the bottom of the dissolution container with the streaming diffusion layer remaining clear as it fell through medium. This indicated that simultaneous solution of both components took place at the solid-liquid interface.

Comparison Between Fluxes of Weak Acid-Phosphate Mixes and Salts of the Weak Acids.—A comparison of fluxes obtained previously for the dissolution of theophylline salts with the maximum flux obtained with the phosphate mixes shows that even the slowest-dissolving theophylline salt, aminophylline, dissolved much faster than the mixture possessing the most rapid rate. This is shown in Table I where the fluxes for the benzoic acid and phenobarbital sodium salts, as well as the ethylenediamine salt, are compared with the maximum rates per unit area obtained using phosphate mixes.

The fluxes listed would be larger if anhydrous sodium phosphate had been used. The fluxes would be increased by a factor of about 1.4 to 1.8 for the compositions with maximum rates. This correction does not increase flux values in Table I for acid-phosphate compositions to equal or exceed the values found for the salts.

Effect of Medium on Rate.—The use of acidic medium could be expected to decrease rate of solution of the weak acid-phosphate mixes. A comparative study is shown on Fig. 1. It will be seen that the depression in rate was more marked with the mixes than with a salt of theophylline. The buffer

TABLE I.—COMPARISON OF SOLUTION RATE PER UNIT AREA VALUES—TRIBASIC SODIUM PHOSPHATE-ACID MIXES vs. SALTS OF ACIDS

Compound or Composition	Rate ^a
Ethylenediamine theophylline (aminophylline)	6.53 ^{b, c}
Theophylline $2/3$ mole-tribasic sodium phosphate $1/3$ mole	2.03 ^{b, c}
Sodium phenobarbital	4.3 ^{b, d}
Phenobarbital $1/2$ mole-tribasic sodium phosphate $1/2$ mole	0.48 ^{b, d}
Sodium benzoate	1.85 ^{d, e}
Benzoic acid $2/3$ mole-tribasic sodium phosphate $1/3$ mole	0.26 ^{d, e}

^a Gm./sec.-cm.² $\times 10^4$ of acid at 25°.

^b Dissolution in water.

^c Dissolution in $0.1\ N$ HCl

^d Dissolution by free convection.

^e Dissolution in stirred mediums.

capacity of the diffusion layer was no doubt greater in the true salt.

Effect of Acid Strength on Amount of Phosphate Required to Obtain Maximum Flux.—An examination of Fig. 2 will show a decrease in amount of phosphate needed to obtain maximum flux with increase in strength of acids. The literature values for pK 's of theobromine, theophylline, and benzoic acid are, respectively, 10.1, 8.77, and 4.19 (7). The acid strength of phenobarbital does not correlate with this grouping, but it was studied by a different process.

SUMMARY

The dissolution rate of weak acids was found to be considerably enhanced by the addition of tribasic sodium phosphate before compression into pellets. The relationship between rate and composition was complex showing a maximum whose location appeared to be related to acid dissociation constant. When the rate of the composition of a given acid-phosphate mixture showing maximum rate was compared to the rate found using the true salt, the latter rate was always greater. This was true when rates were compared from a given constant surface area.

REFERENCES

- (1) Noyes, A. A., and Whitney, W. R., *J. Am. Chem. Soc.*, **19**, 930(1907)
- (2) Nelson, E., *THIS JOURNAL*, **46**, 607(1957)
- (3) Nelson, E., *ibid.*, **47**, 297(1958)
- (4) Comer, J. P., and Hilty, W. W., *THIS JOURNAL*, **43**, 287(1951)
- (5) Miles, J. W., and Englis, D. T., *THIS JOURNAL*, **43**, 589(1954)
- (6) Doub, L., and Vandenbelt, J. M., *J. Am. Chem. Soc.*, **69**, 2714(1947)
- (7) "Merck Index," 6th ed., Merck and Co., Rahway, N. J., 1952.

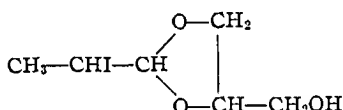
A Study of Certain Physical and I^{131} -Exchange-Tagging Characteristics of Iodopropylidene Glycerol*

By GEORGE F. HOFFNAGLE† and ARTHUR OSOL

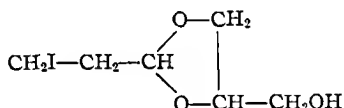
Preparatory to an investigation of the biological distribution and fate of iodopropylidene glycerol in rats, a study of the ultraviolet, visible, and infrared spectrophotometric absorption characteristics of the compound was made, along with a determination of its distribution between water and chloroform. To prepare radioactive iodine-labeled iodopropylidene glycerol suitable for use in the biological study, a process of exchange tagging with radioactive sodium iodide was investigated, and the conditions for obtaining optimum radioactivity in the test material established.

IODOPROPYLIDENE GLYCEROL (hereafter called IPG) has been demonstrated to be a safe and orally efficacious source of iodine for nutritional and therapeutic use in humans (1-4).

The IPG supplied commercially¹ consists principally of 1,2-(2-iodopropylidene)glycerol or 2-(1-iodoethyl)-1,3-dioxolane-4-methanol (2IPG)



with a minor component of 1,2-(3-iodopropylidene) glycerol or 2-(2-iodoethyl)-1,3-dioxolane-4-methanol (3IPG).



No information concerning optical isomers which may be present is available (2IPG has eight possible stereoisomers, 3IPG has four).

The commercial preparation is routinely assayed for IPG content by digestion in a strongly alkaline, aqueous solution at boiling temperature, followed by titration of iodide with silver nitrate V. S. in the presence of nitric acid and a small amount of elemental iodine, with starch T.S. as indicator. The preparations for this study, all of them viscous liquids, contained approximately 34 to 37% of iodine. Since the theoretical content of iodine in the isomeric acetals is 49.2%,

the products supplied contained 70 to 75% of the acetals; the remainder consisted of non-iodinated compounds incident to the synthesis.

The compound 3IPG is considerably more susceptible to decomposition by alkali than 2IPG; because of this it was possible to obtain 2IPG, as well as the mixture. No method for obtaining 3IPG alone was available. Attempts to separate the mixed isomers by distillation were unsuccessful.

EXPERIMENTAL

Concentration of the Acetals; Distribution between Chloroform and Water.—In order to concentrate the acetals present in the preparations provided for these studies, chloroform solutions of the latter were successively extracted with water to remove nonacetal components. The acetals were recovered from the chloroform solutions by distilling the solvent under vacuum with application of mild heat. This procedure was found to be capable of concentrating the iodinated compounds to as high as 92.5%, as shown by alkaline hydrolysis followed by titration of iodide with silver nitrate V.S. The yield of acetals by this extraction procedure was, however, only about 60% of theoretical. The reason for this loss was found after study of the distribution of a 92.5% material between chloroform and water, the distribution ratio for chloroform/water being observed to be approximately 10.

Ultraviolet and Visible Absorption Characteristics.—A spectrophotometric absorption curve (Fig. 1a) in the ultraviolet and visible regions for an ethanol solution of a preparation containing 71% of mixed IPG isomers was prepared from measurements made with a Beckman DU spectrophotometer. A single absorption peak was found at a wavelength of 255 $m\mu$, and a minimum at 233 $m\mu$. The absorptivity (1%, 1 cm.) at 255 $m\mu$ was found to be 19.1 based on a concentration of 366 mg. per liter. Only slight deviation from Beer's Law was observed in the range of concentration from 50 to 450 mg. per liter (Fig. 1b). Other samples agreed within 1.2% based on their IPG content.

Infrared Absorption Characteristics.—Infrared spectrophotometric absorption curves (Fig. 2) were obtained for three samples of the preparation as noted using a Baird instrument. The samples were

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Abstracted in part from a dissertation submitted by George F. Hoffnagle to the Graduate School of the Philadelphia College of Pharmacy and Science in partial fulfillment of the requirements for the degree of Doctor of Science.

This investigation was supported in part by a grant from Henry K. Wampole & Co., Inc., Philadelphia, Pa.

† Present address: 86 Forest Ave., Verona, N. J.

¹ Preparations of IPG used in this study were supplied by Henry K. Wampole & Co., Inc., Philadelphia, Pa.

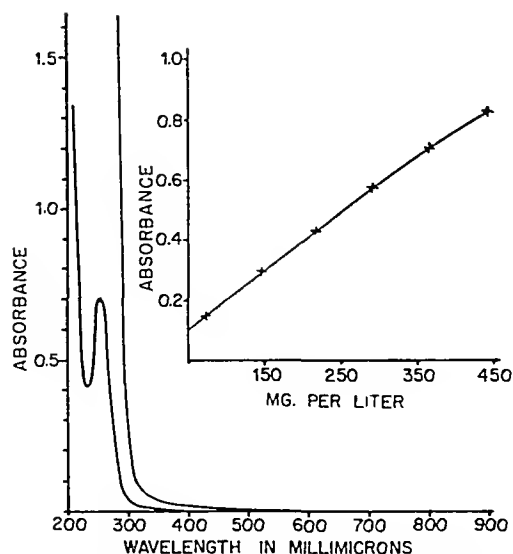


Fig. 1a.—Absorption characteristics of IPG at concentrations of 386 mg. per liter and 3660 mg. per liter in ethanol in the ultraviolet and visible regions.

Fig. 1b.—Variation of absorbance with concentration of IPG in ethanol at a wavelength of 255 mμ.

placed undiluted between sodium chloride plates. By means of these curves, it appears to be possible to distinguish between IPG and compounds incident to the synthesis, and to accept the top curve as a good approximation of the absorption characteristics of IPG. The tentatively identifiable peaks are noted in Fig. 2. The carbonyl peaks can be removed either by mild alkaline treatment or by solvent extraction, and can be estimated by the hydroxylamine reaction; the carbonyl is not in the IPG structure. The hydroxyl peak can be reduced but not removed by concentration procedures, indicating its presence both within and outside the IPG structure. Absorption by the iodine atom is beyond the range of the instrument, but residual chloroform from the concentration procedure is present in the top and center curves. The peaks at $6.19\ \mu$ and in the range of 8 to $10\ \mu$ are common to cyclic ethers such as dioxanes and aliphatic ethers.

Investigation of Tagging of IPG with Iodine-131.

—The ultimate purpose of this phase of the study was to provide a radioactive material of sufficient activity to trace IPG in biological experiments.

Exchange tagging of 2IPG was studied under four conditions. In formulation 1 the IPG and elemental I^{131} were mixed in a mildly alkaline medium; in formulation 2 the IPG and I^{131} as iodide were mixed in a mildly alkaline medium; in formulation 3 the IPG and elemental I^{131} were mixed in a mildly acid medium; in formulation 4 the IPG and I^{131} as iodide were mixed in a mildly acid medium. The compositions of the media used are set forth in Table I.

The solutions were allowed to stand in glass-stoppered bottles at room temperature. To determine the extent of exchange-tagging the following procedure was used. Samples of 20 ml. each were removed from each solution at four, seven, and ten days. The solutions were treated to remove inorganic iodine as follows. Dilution of the inorganic iodine was accomplished by the addition of 100 mg. of potassium iodide. Since the amount of I^{131} at the start of the experiment was approximately 5.4×10^{-7} mg., this dilution amounts to some 1.4×10^5 times. The solution was then made acid with acetic acid, 0.5 ml. of starch T. S. added, and sufficient potassium permanganate T. S., to obtain a blue color. A slurry of silver carbonate was then added dropwise, slowly and with agitation, until the blue color just disappeared. The mixture was diluted to 25 ml. with distilled water and quickly filtered through a Seitz filter, using a K5 pad, thereby removing the silver iodide and any unreacted silver carbonate. This procedure, when followed meticulously, yielded a solution which would neither darken on exposure to light, nor show any precipitate on adding a small amount of sodium chloride. Also, the radioactivity could be reduced to approximately background activity by extracting the solution with four 10-ml. portions of chloroform, thereby indicating that no appreciable portion of the radioactivity was present as iodide. Furthermore, paper chromatograms (5) showed no detectable iodide, iodate, or free iodine. The total iodine content in the final solutions was determined by alkaline digestion followed by titration with 0.01 *N* silver nitrate. The K5 pads were found to have absorbed only an estimated 0 to 5% of IPG from the solutions.

The radioactivity of the sample, diluted with 0.1% sodium iodide solution, was determined using an organic-quenched, end-window, Geiger-Mueller counter tube, the sample being 2 ml. of solution held in a plastic planchet. An equilibrium sample of RaDEF was used as the standard for calibrating the equipment, and the samples were converted to curies of activity, where desired, by comparing with dilutions of the carrier-free sodium iodide- I^{131} supplied by Abbott Laboratories, using the value of the standard

TABLE I.—COMPOSITION OF MEDIA EMPLOYED IN TAGGING IPG WITH I^{131}

Ingredients —	Composition of Formulation			
	1	2	3	4
2IPG	0.15 Gm.	0.15 Gm.	0.15 Gm.	0.15 Gm.
Iodine equivalent	0.0738 Gm.	0.0738 Gm.	0.0738 Gm.	0.0738 Gm.
Glycerin	45 ml.	45 ml.	45 ml.	45 ml.
Distilled water	15 ml.	15 ml.	15 ml.	15 ml.
Sodium bicarbonate	1 Gm.	1 Gm.
Glacial acetic acid	1 ml.	1 ml.
Carrier-free NaI^{131}	0.2 mc.	0.2 mc.	0.2 mc.	0.2 mc.
Potassium permang. T. S.	0.2 ml.	0.2 ml.

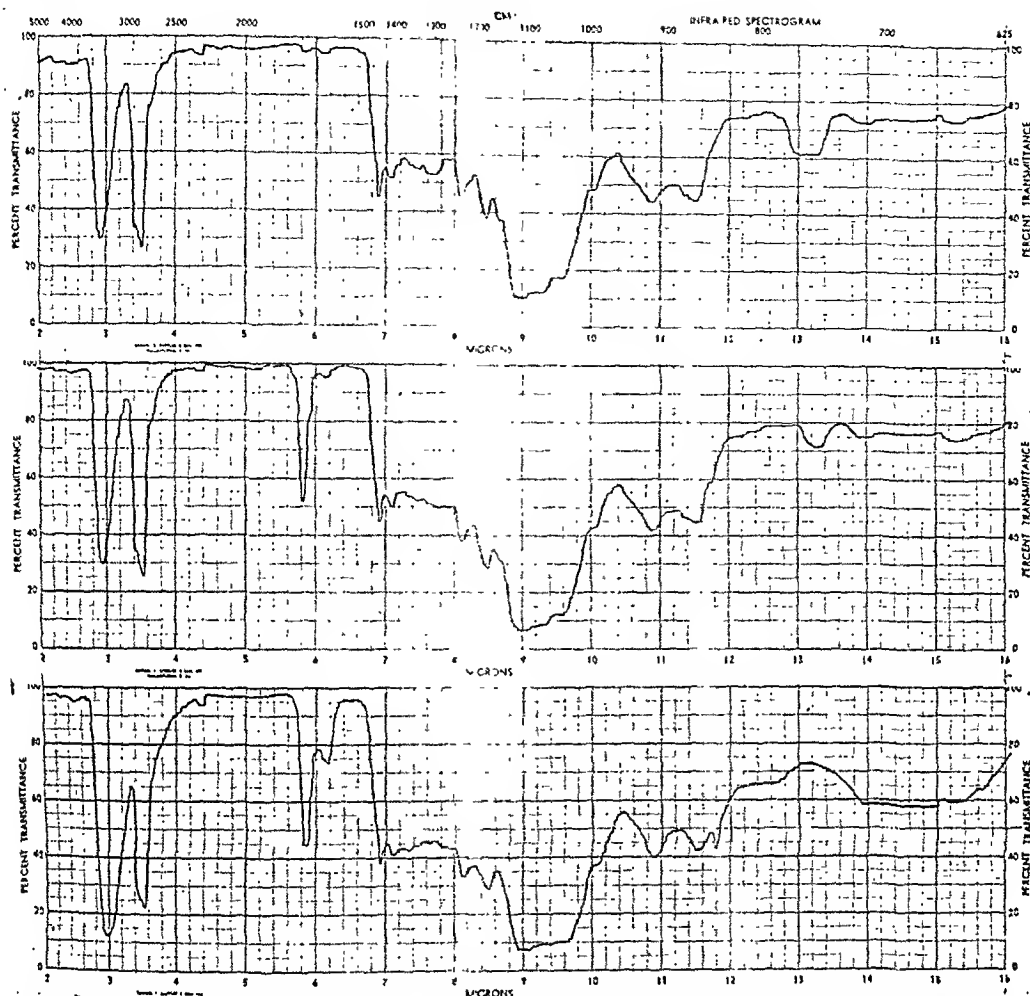


Fig. 2.—Infrared absorption curves of IPG. Top—2IPG at 92.5 per cent purity. Center—Mixed IPG at 85.8 per cent purity. Bottom—Mixed IPG at 71.2 per cent purity.

supplied by them, corrected for decay. The results of the tagging are in Table II.

The same experiments using mixed IPG yielded the same results in acid media, but even lower results in alkaline media because of the lability of 3IPG under these conditions.

TABLE II.—EXTENT OF TAGGING OF IPG WITH I^{131} IN VARIOUS MEDIA, IN MICROCURIES PER MG. OF IODINE IN IPG, NOT CORRECTED FOR DECAY

Exchange Time	Formulation			
	1	2	3	4
4 days	0.264	0.168	0.364	0.291
7 days	0.072	0.143	0.350	0.304

A second type of experiment was undertaken employing the optimal conditions for tagging as represented by formulation #3 above, but adding varying amounts of I^{131} to a constant amount of 2IPG (60 mg.); the amounts used and the results, using the same sampling methods as above, are found in Table III.

TABLE III.—EXTENT OF TAGGING OF IPG USING DIFFERENT INITIAL AMOUNTS OF I^{131} , IN MICROCURIES PER MG. OF IODINE IN IPG, NOT CORRECTED FOR DECAY

Sample	Radioactivity added initially to 60 mg 2IPG	—Exchange Time—		
		36 Hr.	72 Hr	120 Hr
I	0.4 millicurie	0.69	0.86	0.87
II	2.0 millicuries	2.0	2.5	2.5
III	4.0 millicuries	3.0	3.8	4.3

These data clearly pointed to the probability of obtaining even higher specific activities of tagged IPG by increasing the proportion of radioactive sodium iodide to IPG. Further experimentation proved this to occur. However, this exchange reaction could not be characterized as zero-order, first-order, or second-order.

This information was used to produce tagged IPG of high specific activities for chromatographic (5) and biological (6) use. The results of a series of such experiments with varying proportions of NaI^{131} to IPG and varying times are listed in Table IV.

TABLE IV.—¹³¹I TAGGING OF 2IPG TO PRODUCE HIGH SPECIFIC ACTIVITIES, REPORTED AS MICROCURIES PER MG. OF IODINE IN IPG, NOT CORRECTED FOR DECAY

Added radioactivity	9 mc.	9 mc.	8.3 mc.	9 mc.	6.9 mc.
Iodine in 2IPG	53.7 mg	31.6 mg.	34.8 mg.	54.0 mg.	39.8 mg.
Exchange Time	7 days	8 days	12 days	13 days	16 days
Final radioactivity	15.4 uc	23.8 uc.	24.4 uc.	8.0 uc.	7.0 uc.

The raw material ratio and exchange time which produce the highest specific activities are apparent from Table IV, and were employed when such material was required.

REFERENCES

(1) McKnight, R. B., *N. Carolina Med. J.*, 7, 590 (1946).

(2) McKnight, R. B., *Southern Surgery*, 13, 810 (1947).

(3) Fiearra, B. J., *Medical Times*, Oct., 1954.

(4) Slaughter, D., *et al.*, *Am. Therap. Soc. Meeting*, Boston, April 13-16, 1950.

(5) Hoffnagle, G. F., Sideri, C. N., and Osol, A., in press, *THIS JOURNAL*, May, 1958.

(6) Hoffnagle, G. F., and Osol, A., *THIS JOURNAL*, 47, 149 (1958).

Book Notices

Medical Writing, The Technique and the Art, 3rd ed. By MORRIS FISHBEIN. The Blakiston Division, McGraw-Hill Book Co., Inc., New York, 1957. x + 262 pp. 15 x 23 cm. Price \$7.

The sound advice given by the experienced editor and author, Dr. Fishbein, can apply equally well to anyone who is preparing a report for publication. Although the author's experience has been mainly in the field of medical journalism, the terminology, illustrations, etc., utilized in this text are medical. A brief chapter deals with pharmaceutical products and prescriptions. The excellent index is particularly useful. This is a good handbook for any editorial office.

The National Formulary of Japan 2nd ed. Compiled and edited by the Formulary Section of National Council of Pharmacy. English edition published by the Ministry of Health and Welfare, Tokyo, 1957. 14.5 x 21 cm. vii + 167 pp.

The second edition of the N. F. (Japan) is evidence of the progress that has been made in the introduction of new medicinals into the Japanese materia medica, although some folklore medicinals are still noted, e.g. Japanese Ginseng and Oyster Shell. The text monographs are arranged according to the Latin title of the drug or dosage form, and this makes one realize the greater convenience of the arrangement in the N. F. (U. S.). The English title is not always a translation of the Latin; e.g. *Tiglim*, Croton Seed; with the synonym *Tiglii Semen*. *Epirenaminæ bitartras* has the English title Epirenamine Bitartrate, but *Collunarium epirenaminæ compositum* has the English title Compound Epinephrine Nose Drops. Epirenamine is not included among the 36 names for epinephrine that are given in the Merck Index 6th ed.

The monographs represent excellent technical study and preparation, but it is surprising to note

that the formulas and equivalence factors are based on atomic weights adopted by the International Committee in 1949; although the investigative phase of revision was continued into 1954. In view of the difficulty experienced with the use of sodium acetate as the buffer in the N. F. (U. S.) assay for methionine, it is interesting to note that the N. F. (Japan) monograph utilizes the phosphate-acid phosphate buffer, which probably is preferable.

Appendixes give General Notices and General Tests, Processes and Apparatus of The Pharmacopoeia of Japan 6th ed. The Revision Committee is to be congratulated for its excellent work in preparing this edition of the N. F. (Japan).

The Principles of Therapeutics. By J. HAROLD BURN. Charles C Thomas, Publisher, Springfield, Ill., 1957. ix + 278 pp. 14 x 22.5 cm. Price \$5.50.

This book is a compilation of the author's lectures on "Pharmacology and the Basis of Therapeutics." The subjects included are: Development of therapeutics; Ion exchange in cellular activity, active substances in the body; Transmission of nerve impulses by acetylcholine and noradrenaline; Properties of acetylcholine and of substances modifying its action; Treatment of hypertension; Substances whose action resembles that of acetylcholine, especially nicotine; Adrenaline and the sympathomimetic amines; Action of histamine, anaphylaxis, allergy, histamine liberators and antihistamine substances; Hydroxytryptamine and bradykinin; Pituitary (posterior lobe) extract, Biological standardization; Digitalis glycosides; Substances relaxing smooth muscle including vasodilators; Analgesic substances; Cocaine and the local anesthetics; Alcohol; Anesthetics; Barbiturates and tranquilizing drugs; Stimulant drugs; Drugs of the alimentary canal; Action of drugs on

the uterus, ergot; The treatment of anemias; Thyroid Gland; Insulin; Oestrogens androgens; Adrenal cortex; Disinfectants; Sulfonamides and salicylates; Kidney, treatment of oedema; Antibiotics; Malaria; Chemotherapy in tropical diseases; Metallic salts and vitamins; Biological variation and clinical trials. The book is intended for medical students and physicians. It should be a useful addition to the Pharmacy library as well.

Quantitative Organic Analysis. By JAMES S. FRITZ and GEORGE S. HAMMOND. John Wiley & Sons, Inc., New York, 1957. ix + 303 pp. 14.5 x 23 cm. Price \$6.50.

This textbook utilizes the principle of analysis of organic compounds by selective, functional group reactions. The following chapter headings indicate the scope of the text: Details of a method (analytical), Acid-base titrations in nonaqueous solvents, Indirect acid-base methods, Oxidative and reductive methods, Manometric methods, Metal ion complexes, Elemental analysis, Kinetics in analysis, Spectrophotometric methods, Separations, Physical and polarographic methods, Solving new analytical problems, and Laboratory procedures. The text is designed for use at the later undergraduate and graduate level of instruction. It is documented throughout with references to the literature. Problems are included at the end of each chapter. Author and subject indexes are appended. The book should be considered by teachers of organic analysis and newer analytical techniques.

Recent Progress in Hormone Research, Vol. XIII: Proceedings of the Laurentian Hormone Conference 1956. Edited by GREGORY PINCUS. Academic Press Inc., New York, 1957. v + 646 pp. 15 x 23 cm. Price \$12.80.

The papers and discussions of the thirteenth annual Laurentian Hormone Conference comprise this volume. The twelfth volume was reviewed in *THIS JOURNAL*, 46, 149(1957). The reports, grouped under five main headings, are: I. Neurohumoral-Endocrine Relationships.—Biochemical, physiological, and pharmacological aspects of serotonin; Effects of midbrain and spinal cord transection on endocrine and metabolic functions with postulation of a midbrain hypothalamico-pituitary activating system; Studies on the influence of the central nervous system on anterior pituitary function; Hormones and rhythms in man and animals. II. Hormone Transport in Circulation.—Interaction of thyroid hormones and protein in biological fluids; Binding of steroids and steroid conjugates to human plasma proteins. III. Aspects of Reproduction.—Some experimental studies on the Mechanism of ova-implantation in the rat; Synthetic progestins in the normal human menstrual cycle; Metabolism of progesterone and its clinical use in pregnancy; Long-acting steroids in reproduction. IV. Hormone Chemistry and Metabolism.—Insulinase, insulinase-inhibitors, and diabetes mellitus; Glucagon, a second pancreatic hormone. V. Hormones and Stress.—Endocrine changes after anesthesia, surgery, and unanesthetized trauma in man; Adrenal influences upon the stomach and the gastric responses to stress.

The excellent style, format, and binding of this series is continued. The references at the end of each paper and the appended author and subject indexes add to the value of this volume, and the other volumes in this series, as additions to the reference library in this field.

The Lipids. Their Chemistry and Biochemistry. Vol. 3, Biochemistry (Biosynthesis, Oxidation, Metabolism, and Nutritional Value). By HARRY J. DEUEL, JR. Interscience Publishers, Inc., New York, 1957. xxxvi + 1065 pp. 15.5 x 23.5 cm. Price \$25.

This is the third volume of a comprehensive text on the lipids, and completes the coverage of the biochemical phase of the subject. The author, Dr. Deuel, died in 1956, and some of the later material was added by an editorial committee. The fine style, detailed scope, and excellent printing and format of this volume are similar to that in vols. 1 and 2 which were reviewed, respectively, in *THIS JOURNAL*, 41, 57(1952) and 44, 318(1955). Of particular interest at this time is the final chapter on the nutritional value of fats. The many references throughout the text and the extensive author and subject indexes make this set of books indispensable as a reference work.

Experimental Control of Plant Growth. By FRITS W. WENT. Chronica Botanica Co., Waltham, Mass., 1957. xvii + 343 pp. 15 x 23 cm. Price \$8.50.

This book presents in detail the factors affecting plant growth and describes the methods used in the experimental studies at the Clark Greenhouses and the Earhart Plant Research Laboratory. In addition to author and subject indexes, 27 pages of photographs are appended.

Practical Clinical Chemistry: A Guide for Technicians, 2nd ed. By ALMA HILLER. Charles C Thomas, Springfield, 1957. xvii + 265 pp. 16 x 23.5 cm. Price \$6.50.

The various tests and procedures utilized in clinical chemistry are described in detail and outlines for the different determinations are included. Duplicate forms of the outlines are appended for removal from the book. A general subject index is included. The book should be a useful textbook and laboratory manual as well as a guide for practicing clinical technicians.

Cortisone Therapy: Mainly Applied to the Rheumatic Diseases. By J. H. GLYN. Philosophical Library, Inc., New York, 1957. x + 162 pp. 14 x 22 cm. Price \$10.

The relative therapeutic value of cortisone, corticotrophin, hydrocortisone, prednisone, and prednisolone, primarily in the treatment of rheumatic diseases are reviewed, with detailed regimens of treatment included. It is an important contribution to the rational consideration of a complicated problem in medical practice by a practitioner who is personally familiar with the practical aspects of this field of therapy. A general index is appended.

Actualités Pharmacologiques Series No 10 RENE HAZARD, Editor Masson et Cie, Paris 6, France, 1957 16 5 x 25 4 cm 269 pp

This tenth volume of the series (in French) contains contributed chapters on Mode of action of substances protecting living bodies against ionizing radiations, Chemical mediators and central nervous activities in vertebrates, Organophosphorus anticholinesterases, Pharmacologic action of spinally administered medicaments, Metabolic transformations of organic toxic substances (general biology and industrial hygiene), Pharmacodynamics and comparative physiology, Sulfamide inhibitors of tubular functions, inhibitors of carbonic anhydrase, and hypoglycemic agents, Human plasma fractions and their therapeutic uses. The paper and printing are good, but the volume has a paper back. An alphabetical index of authors of chapters in vols 1-10 is appended.

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Klink und Therapie der Vergiftungen By SVEN MOESCHLIN Georg Thieme Verlag, Stuttgart, 1956 Interccontinental Medical Book Corporation, New York vi + 521 pp 18 5 x 25 cm Price DM 59 Price \$14 05

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ter appears to be arranged in a logical order. It begins with discussions of the frequency and distribution of poisoning, general principles employed in the treatment of poisoning, and a listing of the more important materials and preparations used in the treatment of cases of poisoning. These general chapters are followed by a useful table of maximum allowable concentrations, expressed in parts per million, of gases, vapors, and dusts in the air to which workers may be exposed without creating a health hazard through inhalation of the potential poisons listed. Proceeding from this table the book is divided into sections, each with several subdivisions, relating to inorganic chemicals, organic chemicals, vitamins (A, B, C, D), hormones, poisons from plant sources and the derivatives, antibiotics, food poisons, and animal poisons. Sources of exposure are then discussed.

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NUMBER 5

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By OSCAR E. ARAUJO† and PATRICK F. BELCASTRO

Data are presented showing the influence of insonation intensity, time of exposure, concentration of suspended material, and addition of dispersing agents on the particle size and rate of sedimentation of certain medicaments. All suspensions exposed to ultrasonics have a smaller particle size diameter, a slower rate of sedimentation, and are more stable than the control or noninsonated suspensions.

THE EFFECTS of ultrasonics on matter can be attributed to cavitation, tremendous mechanical forces, heat, depolymerization, and other factors. The first significant study of the thermal, biological, and chemical effects of ultrasonic vibrations was reported by Wood and Loomis (1). Myers and Goodman (2) applied the mechanical forces of this energy in devising an ultrasonic homogenizer, the "Rapisonic," which produced a calamine lotion stable for three months. Recently, Misek and Skauen (3) reported certain effects of ultrasonics on pharmaceutical dispersions. Other investigators (4-7) have studied the value of ultrasound as a means of dispersing solids in a liquid medium.

This work is an attempt to evaluate quantitatively certain pharmaceutical suspensions which have been exposed to the mechanical forces of ultrasonics with the ultimate purpose of producing preparations that are superior to control or noninsonated samples. The rate of sedimentation and the particle size diameters were the

parameters chosen for evaluating the suspensions mentioned in this paper.

APPARATUS

The apparatus used in this study consisted of a 250-watt radio-frequency generator with a variable frequency range of 100 to 1000 kilocycles per second (8). The Hypersonic Transducer Model BU-305 used in conjunction with the generator operates at a frequency of 400 kilocycles per second. The transducer contains a concave bowl of barium titanate which focuses the sound energy for an area of 0.5 square cm. at a point 57.4 mm. above the center of the element.

EXPERIMENTAL

The insonation chamber used in this investigation consisted of a 25 x 150 mm. lipless pyrex test tube, to which was adapted a glass water-jacket so that cold water could be continuously circulated to cool the suspension during insonation.

A volume of 20 ml. of suspension was employed throughout the study. Four insoluble medicaments commonly administered in the form of aqueous suspensions were selected: zinc oxide, bismuth subcarbonate, sulfathiazole, and procaine penicillin G. These substances are representative ingredients of external, oral, and parenteral pharmaceutical suspensions.

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TABLE II.—PARTICLE SIZE OF INSONATED AND NONINSONATED ZINC OXIDE SUSPENSIONS

Concentration of Zinc Oxide	ZnO in Distilled H ₂ O		ZnO with 0.1% CMC		ZnO in 0.1% Marasperse C ^c	
	<i>d</i> _{av.} ^a	<i>s</i> ^b	<i>d</i> _{av.}	<i>s</i>	<i>d</i> _{av.}	<i>s</i>
2% (Control)	8.5	6.5	14.5	9.6	1.8	3.5
5% (Control)	22.1	18.5	19.8	10.9	1.2	2.2
10% (Control)	26.9	8.2	17.3	11.0	1.5	3.4
2% (Insonated)	1.2	1.6	0.85	0.94	0.58	0.24
5% (Insonated)	1.9	3.5	9.6	9.6	0.64	0.36
10% (Insonated)	26.9	9.9	12.9	11.0	0.71	0.50

^a Arithmetic mean diameter in microns.^b Standard deviation calculated from the formula: $s = \sqrt{\frac{\sum nd^2}{\sum n} - d_{av.}^2}$ ^c Marathon Corporation, Rothschild, Wis.

TABLE III.—PARTICLE SIZE OF INSONATED AND NONINSONATED BISMUTH SUBCARBONATE SUSPENSIONS

Concentration of Bi Subcarbonate	In Distilled Water		With 0.1% CMC		With 0.05% CMC and Marasperse C	
	<i>d</i> _{av.} ^a	<i>s</i> ^b	<i>d</i> _{av.}	<i>s</i>	<i>d</i> _{av.}	<i>s</i>
2% (Control)	7.3	7.2	4.9	4.7	5.3	4.9
5% (Control)	8.1	7.8	5.5	8.2	5.2	6.0
10% (Control)	9.0	8.6	4.8	5.0	5.2	5.1
2% (Insonated)	4.0	5.1	3.4	3.3	3.8	4.5
5% (Insonated)	3.8	5.4	4.5	4.4	3.4	2.8
10% (Insonated)	4.5	4.2	4.0	3.6	4.5	4.6

^a Arithmetic mean diameter in microns.^b Standard deviation calculated from the formula: $s = \sqrt{\frac{\sum nd^2}{\sum n} - d_{av.}^2}$

A similar comparison of the bismuth subcarbonate suspensions was impossible, since the suspension insonated at maximum conditions never reached its "half-life" point during the course of these experiments. However, at the 30% point in the decrease of the absorbance, the rate of sedimentation ratio observed was 1 to 17.5. This indicated, following the previously mentioned reasoning, that the particle size of the medicament in the control suspension was 4.2 times larger than that of the insonated suspension.

The sulfathiazole suspensions thus evaluated showed a sedimentation ratio of 1 to 11.6, and the particle size of the solid in the control suspension was 3.4 times larger than that of the insonated suspension.

The procaine penicillin G suspensions exhibited a sedimentation ratio of 1 to 4.6, and the particle size of the suspended medicament in the control suspension was 2.1 times greater than that in the insonated suspension.

From these data, it can be seen that the maximum effect of ultrasonics was observed with the bismuth subcarbonate suspensions, followed in order by sulfathiazole, zinc oxide, and procaine penicillin G.

It must be noted, however, that at maximum conditions of insonation both sulfathiazole and procaine penicillin G suspensions showed some evidence of decomposition, exhibiting a change in color and developing a disagreeable odor.

Effect of Ultrasonics on Concentrated Suspensions.—At this point in the study, it was desired to evaluate the effect of ultrasound on more concentrated suspensions of the previously mentioned compounds. Since it was evident from the foregoing results that the greatest effect was evidenced when the suspensions were subjected to insonation at an intensity of 110 (estimated at 365 watts) for a period of fifteen minutes, these conditions were used exclusively in this phase of the investigation. Since sulfathiazole and procaine penicillin G previously showed evidence of decomposition, further investigation of these suspensions was abandoned.

The spectrophotometric method previously used to evaluate the suspensions not only was impractical for high concentrations, but provided only relative particle size relationships by means of an indirect parameter, such as absorbance. Thus, it became necessary to find a method that would provide an entire particle size distribution of the solids in suspension and that would also furnish data for calculation of absolute mean diameters of the particles. A liquid elutriation method was investigated, but it proved to be insufficiently sensitive to classify particles smaller than 10 microns. Finally the Andreasen pipet, as described by Schweyer (9) produced satisfactory results. This apparatus operates on the principle that if samples are taken from a constant level near the bottom of a cylinder containing a suspension, at appropriate intervals of time, Stokes' law can be applied and the particle size distribution of the suspension can be calculated.

Since increasing the concentration of any solid material in a liquid medium results in a greater tendency towards particle flocculation in the suspension, a small amount of a protective colloid and a dispersing agent was added. Three different media were therefore used: distilled water, distilled water and 0.1% sodium carboxymethylcellulose

(CMC), and distilled water and 0.1% Marasperse C. In the case of bismuth subcarbonate, a combination of 0.05% CMC and 0.05% Marasperse C in distilled water was used in lieu of 0.1% Marasperse C, because better initial dispersion was thus obtained. Three concentrations of the solid materials were used: 2, 5, and 10%.

Figures 1, 2, and 3 show sample plots of the particle size distribution obtained from the data. Tables II and III indicate the relative effect of

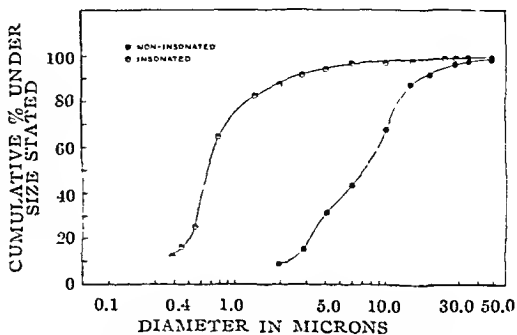


Fig. 1.—Particle size distribution of 2% zinc oxide suspensions in distilled water.

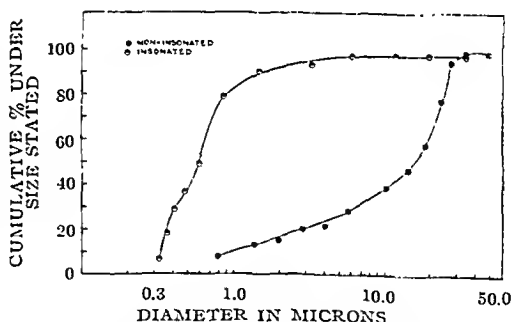


Fig. 2.—Particle size distribution of 2% zinc oxide suspensions containing 0.1% CMC.

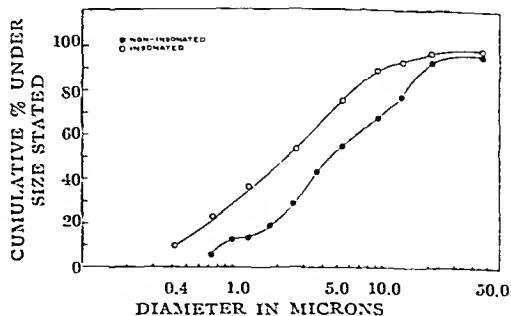


Fig. 3.—Particle size distribution of 2% bismuth subcarbonate suspensions in distilled water.

Effect of Ultrasonics on Dilute Suspensions.—A spectrophotometric method was developed to evaluate the relative effect of ultrasonic energy on the rate of sedimentation of the previously mentioned medicaments. The suspensions were simple systems of the substance in distilled water. They were exposed to ultrasonics for periods of either 5, 10, or 15 minutes. Ultrasonic intensities at rheostat settings of 30, 70, and 110 were employed, representing energies estimated at 28.5, 119, and 365 watts, respectively. However, since ultrasound concentrates the sound waves, the actual energy at the focal point may far exceed the above values.

The Beckman Model B spectrophotometer was employed to determine the absorbance of the suspensions. All experiments were carried out at a wavelength of 500 μ . Absorbance can be directly related to the rate of sedimentation of solid particles in suspension, this rate being a function of the particle size of the solid material. As the particles settle out of the light path, the absorbance decreases at a rate inversely proportional to the size of the particles in suspension.

Since it was impractical to extend the experimentation period to a point where all the particles were no longer in the light path, all absorbance values were measured at intervals of time, only until the initial absorbance was reduced to 50%. In this manner, a partial rate of sedimentation was determined which excluded only the very small particles.

Theinsonated suspensions were compared to control suspensions to observe differences in rate of settling due to the effect of ultrasonic vibrations. The concentration of the solid used is restricted by the quanta of light that can be transmitted through

the suspension, to give a reading on the spectrophotometer, thus limiting this phase of the work to the use of dilute suspensions.

Table I shows the rate of sedimentation of the suspensions studied.

The results clearly indicated the effect of increasing intensity of ultrasonics. In the case of all four substances, a progressively greater reduction in particle size, as evidenced by retardation of the rate of sedimentation, occurred with a corresponding increase in ultrasonic intensity.

By the same token, as the time of exposure to ultrasound was prolonged, the rate of sedimentation of the solid particles in all suspensions was correspondingly retarded.

These facts would lead to the conclusion that the longer the period of insonation and the higher the intensity of ultrasound, the more pronounced is the reducing effect on the particle size of the suspended solids.

However, the data from the tables show that the extent of the effect of ultrasonics varied with each compound. A comparison between the time required for a control suspension of zinc oxide to reach the "half-life" point (50% decrease in absorbance), and the time required for the suspension insonated at maximum conditions of exposure and intensity to reach the corresponding stage, showed that the rate of sedimentation ratio was 1 to 4.9.

Since, according to Stokes' law, the rate of sedimentation varies directly with the square of the diameter of the particle, it seemed logical to hypothesize that the particle size of the solid in the control suspension was 2.2 times as large as that in the suspension insonated at maximum conditions of exposure and intensity.

TABLE I — EFFECT OF ULTRASONICS ON THE SEDIMENTATION RATE OF VARIOUS MEDICAMENTS

% Decrease in Ab- sorbance	Non- inso- nated	Inten- sity of 30 for 5 min	Inten- sity of 30 for 10 min	Inten- sity of 30 for 15 min	Inten- sity of 70 for 5 min	Inten- sity of 70 for 10 min	Inten- sity of 70 for 15 min	Inten- sity of 110 for 5 min	Inten- sity of 110 for 10 min	Inten- sity of 110 for 15 min
Zinc Oxide Suspension 0.03%										
5	2.9	5.0	6.1	6.5	14.1	19.8	30.2	21.2	23.2	33.1
20	9.1	19.1	23.8	27.3	32.3	35.4	46.2	40.4	39.3	62.4
30	13.2	25.4	31.6	35.2	41.8	45.2	57.5	50.7	51.2	76.1
50	22.4	38.8	47.5	53.8	64.9	71.9	87.4	79.1	88.9	108.7
Bismuth Subcarbonate Suspension 0.03%										
	0.7	1.5	1.6	1.9	3.1	2.7	3.6	4.2	4.1	4.9
20	3.1	6.5	7.0	9.1	13.0	10.6	14.4	20.6	27.3	41.5
30	5.6	11.5	16.4	16.8	23.0	20.0	30.8	36.2	52.4	98.0
50	14.8	46.6	58.4	62.4	74.3	78.1	101.4	121.7	"	"
Sulfathiazole Suspension 0.5%										
5	0.2	1.3	1.5	1.4	1.6	1.4	1.4	1.8	2.9	1.8
20	1.1	4.4	5.6	6.3	8.2	6.4	8.1	9.5	13.4	11.7
30	1.8	6.7	8.3	9.5	13.1	11.4	14.7	17.8	22.0	21.0
50	4.3	12.3	14.8	18.5	24.4	27.3	32.8	42.1	48.1	50.0
Penicillin Procaine G Suspension 1.0%										
5	0.5	0.6	0.7	0.6	0.6	0.7	0.8	0.7	1.1	1.2
20	1.8	2.2	2.2	2.4	2.2	3.0	3.0	2.8	4.2	5.0
30	2.8	3.5	3.5	4.2	3.9	4.8	5.5	5.4	7.5	9.6
50	5.7	8.9	10.5	11.5	12.2	13.5	15.8	19.2	20.9	26.3

* Measurements not taken due to indefinitely long periods of sedimentation.
† Obtained through the courtesy of Chas. Pfizer and Co. Inc., Brooklyn 6, N. Y.

A similar comparison of the bismuth subcarbonate suspensions was impossible, since the suspension insonated at maximum conditions never reached its "half-life" point during the course of these experiments. However, at the 30% point in the decrease of the absorbance, the rate of sedimentation ratio observed was 1 to 17.5. This indicated, following the previously mentioned reasoning, that the particle size of the medicament in the control suspension was 4.2 times larger than that of the insonated suspension.

The sulfathiazole suspensions thus evaluated showed a sedimentation ratio of 1 to 11.6, and the particle size of the solid in the control suspension was 3.4 times larger than that of the insonated suspension.

The procaine penicillin G suspensions exhibited a sedimentation ratio of 1 to 4.6, and the particle size of the suspended medicament in the control suspension was 2.1 times greater than that in the insonated suspension.

From these data, it can be seen that the maximum effect of ultrasonics was observed with the bismuth subcarbonate suspensions, followed in order by sulfathiazole, zinc oxide, and procaine penicillin G.

It must be noted, however, that at maximum conditions of insonation both sulfathiazole and procaine penicillin G suspensions showed some evidence of decomposition, exhibiting a change in color and developing a disagreeable odor.

Effect of Ultrasonics on Concentrated Suspensions.—At this point in the study, it was desired to evaluate the effect of ultrasound on more concentrated suspensions of the previously mentioned compounds. Since it was evident from the foregoing results that the greatest effect was evidenced when the suspensions were subjected to insonation at an intensity of 110 (estimated at 365 watts) for a period of fifteen minutes, these conditions were used exclusively in this phase of the investigation. Since sulfathiazole and procaine penicillin G previously showed evidence of decomposition, further investigation of these suspensions was abandoned.

The spectrophotometric method previously used to evaluate the suspensions not only was impractical for high concentrations, but provided only relative particle size relationships by means of an indirect parameter, such as absorbance. Thus, it became necessary to find a method that would provide an entire particle size distribution of the solids in suspension and that would also furnish data for calculation of absolute mean diameters of the particles. A liquid elutriation method was investigated, but it proved to be insufficiently sensitive to classify particles smaller than 10 microns. Finally the Andreasen pipet, as described by Schweyer (9) produced satisfactory results. This apparatus operates on the principle that if samples are taken from a constant level near the bottom of a cylinder containing a suspension, at appropriate intervals of time, Stokes' law can be applied and the particle size distribution of the suspension can be calculated.

Since increasing the concentration of any solid material in a liquid medium results in a greater tendency towards particle flocculation in the suspension, a small amount of a protective colloid and a dispersing agent was added. Three different media were therefore used: distilled water, distilled water and 0.1% sodium carboxymethylcellulose

(CMC), and distilled water and 0.1% Marasperse C. In the case of bismuth subcarbonate, a combination of 0.05% CMC and 0.05% Marasperse C in distilled water was used in lieu of 0.1% Marasperse C, because better initial dispersion was thus obtained. Three concentrations of the solid materials were used: 2, 5, and 10%.

Figures 1, 2, and 3 show sample plots of the particle size distribution obtained from the data. Tables II and III indicate the relative effect of

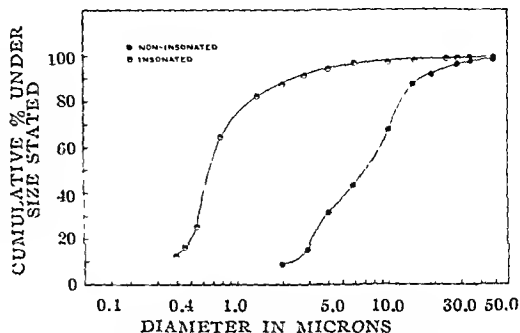


Fig. 1.—Particle size distribution of 2% zinc oxide suspensions in distilled water.

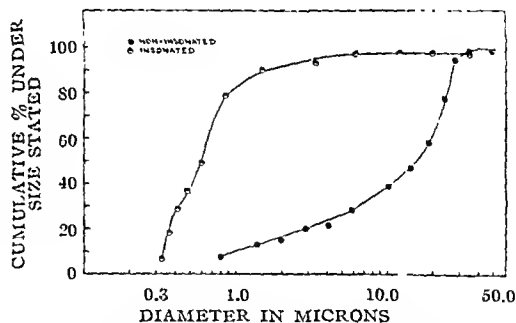


Fig. 2.—Particle size distribution of 2% zinc oxide suspensions containing 0.1% CMC.

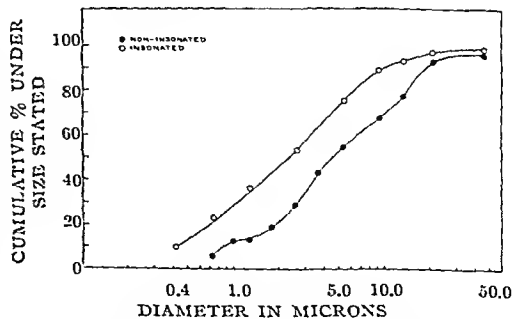


Fig. 3.—Particle size distribution of 2% bismuth subcarbonate suspensions in distilled water

TABLE II.—PARTICLE SIZE OF INSONATED AND NONINSONATED ZINC OXIDE SUSPENSIONS

Concentration of Zinc Oxide	ZnO in Distilled H ₂ O		ZnO with 0.1% CMC		ZnO in 0.1% Marasperse C ^c	
	dav. ^a	s ^b	dav.	s	dav.	s
2% (Control)	8.5	6.5	14.5	9.6	1.8	3.5
5% (Control)	22.1	18.5	19.8	10.9	1.2	2.2
10% (Control)	26.9	8.2	17.3	11.0	1.5	3.4
2% (Insonated)	1.2	1.6	0.85	0.94	0.58	0.24
5% (Insonated)	1.9	3.5	9.6	9.6	0.64	0.36
10% (Insonated)	26.9	9.9	12.9	11.0	0.71	0.50

^a Arithmetic mean diameter in microns.^b Standard deviation calculated from the formula: $s = \sqrt{\frac{\sum nd^2}{\sum n} - \text{dav.}^2}$ ^c Marathon Corporation, Rothschild, Wis.

TABLE III.—PARTICLE SIZE OF INSONATED AND NONINSONATED BISMUTH SUBCARBONATE SUSPENSIONS

Concentration of Bi Subcarbonate	In Distilled Water		With 0.1% CMC		With 0.05% CMC and Marasperse C	
	dav. ^a	s ^b	dav.	s	dav.	s
2% (Control)	7.3	7.2	4.9	4.7	5.3	4.9
5% (Control)	8.1	7.8	5.5	8.2	5.2	6.0
10% (Control)	9.0	8.6	4.8	5.0	5.2	5.1
2% (Insonated)	4.0	5.1	3.4	3.3	3.8	4.5
5% (Insonated)	3.8	5.4	4.5	4.4	3.4	2.8
10% (Insonated)	4.5	4.2	4.0	3.6	4.5	4.6

^a Arithmetic mean diameter in microns^b Standard deviation calculated from the formula: $s = \sqrt{\frac{\sum nd^2}{\sum n} - \text{dav.}^2}$

ultrasonics on the various particle diameters of the suspended medicaments. The zinc oxide suspensions in distilled water showed that as the concentration of the solid was increased, the mean diameter of the particles increased. This effect was minimized by the addition of CMC and was not observed with the suspensions containing Marasperse C. This would indicate that the addition of these agents prevent initial flocculation of the particles, thus permitting ultrasonics to exert its reducing effect, irrespective of concentration. One possible explanation is that by keeping the particles in a deflocculated form, these agents permit the ultrasonic waves to reach the individual particles more readily and thereby exert their reducing effect. However, in all cases, the mean particle size of the solids in the control suspensions was larger than the corresponding insonated suspensions, justifying the original premise that ultrasound would break up suspended particles.

There did not seem to be any significant difference in the effect of concentration on the bismuth subcarbonate suspensions, even with those in plain distilled water. The chemical and physical structure of this compound seems to make it less prone to particle flocculation than was the case with zinc oxide. However, all suspensions showed a reduction in particle size when subjected to ultrasonics.

SUMMARY

- 1 The effect of ultrasonic intensity and time

of insonation on dilute suspensions of four commonly used medicaments was studied.

2. The effect of ultrasonic vibrations on more concentrated suspensions of zinc oxide and bismuth subcarbonate was reported.

3. The influence of the addition of a suspending agent (CMC) and a deflocculating agent (Marasperse C) to suspensions of zinc oxide and bismuth subcarbonate was observed.

4. In all cases, ultrasonic energy reduces the particle size of the suspended medicament and retards its rate of sedimentation, providing a more stable preparation.

REFERENCES

- (1) Wood, R. W., and Loomis, A. L., *Phil. Mag.* [Series 7], 4, 417(1927).
- (2) Myers, J. A., and Goodman, J. E., *Pharm. J.*, 119, 422 (1954).
- (3) Miskel, B., and Skauen, D. M., *THIS JOURNAL*, 47, 32 (1958).
- (4) Sollner, K., *Trans. Faraday Soc.*, 34, 1170(1938).
- (5) Mathieu-Sicaud, A., and Levavasseur, G., *Compt. rend.*, 227, 196(1918).
- (6) Mathieu-Sicaud, A., and Levavasseur, G., *ibid.*, 228, 393(1919).
- (7) Meyer, F., *Die Pharmazie*, 5, 477(1950).
- (8) Judkins, J. R., "A 250 Watt Radio Frequency Generator with a Variable Frequency Range of 100 Kilocycles per Second to 1.0 Megacycles," M.S. Thesis, Purdue University (1951).
- (9) Schweyer, H. E., *Bull. Florida Univ. Eng. Ind. Exp. Station*, 6, No. 6(June 1952).

Anti-inflammatory Effects of Romilar CF*

By LOWELL O. RANDALL and JOSEPH J. SELITTO

Analgesic, antiedematous, and antipyretic effects of Romilar, N-acetyl-*p*-aminophenol, *l*-phenylephrine, chlorpheniramine and a mixture of these ingredients have been demonstrated on the inflamed foot of the rat. Codeine has primarily a central analgesic effect; salicylate and acetophenetidin have primarily a peripheral analgesic, antipyretic, and antiedematous effect while Romilar, N-acetyl-*p*-aminophenol, phenylephrine, and chlorpheniramine and a mixture of these ingredients have mixed central and peripheral effects.

ROMILAR® (DEXTROMETHORPHAN) is a cough suppressant which has the clinical antitussive effectiveness of codeine (1). Romilar® does not have the potent analgesic effects and addictive properties of narcotics (2-4). In order to provide stronger analgesic properties and more decongestant properties, a mixture of Romilar®, chlorpheniramine, *l*-phenylephrine and N-acetyl-*p*-aminophenol has been prepared. The analgesic-antipyretic effectiveness of N-acetyl-*p*-aminophenol was discovered by Flinn and Brodie (5) and confirmed by Boreus and Sandberg (6) and Wallenstein and Houde (7). This paper is concerned with the comparison of the analgesic, antipyretic, and antiedematous effects of the various ingredients alone and the complementary effectiveness of the ingredients in a mixture.

EXPERIMENTAL

Inflammation in the rat's foot was induced by the injection of 0.1 ml. of a 20% suspension of brewer's yeast into the plantar surface. Thresholds to pain in the inflamed and control feet were measured by the pressure method of Randall and Selitto (8). The circumferences of the control and inflamed feet were measured with a loop of string attached to a millimeter scale according to the procedure of Selitto and Randall (9). Temperatures of the feet and the skin of the back were measured with the Universal Thermometer manufactured by Electro Laboratory of Copenhagen.

Drugs were injected subcutaneously into five rats at each dose level and five controls were used. The changes in pain threshold, size, and temperature of both inflamed and control feet, and of skin temperature were measured at intervals of 1, 2, and 4 hours after the administration of the drug. The degree of analgesia was calculated as the increase in mm. Hg in the pain threshold of treated animals in comparison with controls. Temperature changes of the feet and skin were recorded as the difference in degrees centigrade between treated and control animals. Antiedematous effects were calculated as the per cent inhibition of the swelling of the feet of treated animals in comparison with the control animals.

RESULTS AND DISCUSSION

Analgesic effects as indicated by the increase in the pain threshold of the inflamed foot were evident with Romilar®, N-acetyl-*p*-aminophenol, *l*-phenylephrine, chlorpheniramine, and the mixture of these ingredients. Also analgesic effects were observed with salicylate, acetophenetidin and codeine. The relative order of decreasing potency calculated as the dose in mg./Kg. which induced an increase in threshold of 120 mm. Hg was as follows: chlorpheniramine 1, *l*-phenylephrine 8, codeine 10, Romilar® 10, salicylate 100, acetophenetidin 200, and N-acetyl-*p*-aminophenol 400.

The Romilar® CF induced an increase in the pain threshold also. A dose of 2 cc./Kg. orally of the mixture raised the threshold over 100 mm. Hg. This dose contained 6 mg. of Romilar®, 0.5 mg. of chlorpheniramine, 2 mg. of *l*-phenylephrine, 48 mg. of N-acetyl-*p*-aminophenol. As tested on inflamed tissue this combination had an antipyretic-analgesic effectiveness approximately equal to the narcotic analgesic effects of 10 mg. of codeine.

Significant analgesic effects as measured on the pain threshold of normal noninflamed foot was observed only with relatively high doses of codeine, Romilar®, N-acetyl-*p*-aminophenol, *l*-phenylephrine, chlorpheniramine, and the mixture. These effects appeared only at doses 2 to 4 times those which were effective on the pain threshold of the sensitive inflamed foot.

Coincident with the elevation of pain threshold of the inflamed foot, there is reduction of swelling and temperature of the foot with all compounds except codeine. These results are interpreted as indicating that only codeine has true central analgesic effects and lacks any peripheral antipyretic and antiedematous effects. In contrast, acetophenetidin and salicylate have primarily a peripheral action in that they elevate the pain threshold and reduce edema and temperature of the inflamed foot without affecting the pain threshold of the normal foot. The mixed effects of Romilar®, N-acetyl-*p*-aminophenol, *l*-phenylephrine, chlorpheniramine, and the Romilar® CF mixture in elevating pain thresholds, reducing edema and lowering temperature indicates a mixed central and peripheral effect of these compounds. There is the possibility that the peripheral anti-inflammatory effects of these compounds could partially be explained on a circulatory basis also. The permeability of inflamed tissues could readily be changed either by an increase or a decrease in circulation rates and pressures. Under the experi-

* Received August 20, 1957, from the Department of Pharmacology, Hoffmann-La Roche, Inc., Nutley 10, N. J.

TABLE I.—ANTI-INFLAMMATORY EFFECTS IN RATS

Compound	Dose, mg./Kg.	Route	Time Hr.	Analgesia		Anti- edema % Inhi- bition	Antipyresis		
				Infl. Foot mm. Hg	Normal Foot mm. Hg		Infl. Foot ° C.	Normal Foot ° C.	Skin ° C.
Romilar ^r	6.25	P. O.	1	58	0	0	-1.6	-0.2	-0.1
			4	10	0	0	0	0	0
Romilar [®]	12.5	P. O.	1	130	26	13	-1.9	-0.2	-0.3
			4	42	0	0	-0.6	-0.1	0
Romilar ^f	25.0	P. O.	1	232	123	23	-2.3	+0.4	+0.1
			4	210	95	20	-0.6	0	0
N-acetyl- <i>p</i> -aminophenol	100.0	P. O.	1	4	0	0	0	0	0
			4	0	0	0	0	0	0
N-acetyl- <i>p</i> -aminophenol	200.0	P. O.	1	100	32	14	-1.6	0	-0.4
			4	0	0	0	0	0	0
N-acetyl- <i>p</i> -aminophenol	400.0	P. O.	1	235	123	44	-3.9	-1.7	-2.0
			4	220	115	30	-1.4	-0.9	-1.6
Acetophenetidin	200.0	P. O.	1	109	36	18	-1.3	-0.2	-1.0
			4	0	20	0	+0.1	+0.7	-0.4
<i>l</i> -phenylephrine	2.5	P. O.	1	8	0	0	+0.2	+0.1	+0.4
			4	0	0	0	0	0	0
<i>l</i> -phenylephrine	5.0	P. O.	1	71	23	0	-0.6	-0.7	-0.4
			4	7	0	0	0	0	0
<i>l</i> -phenylephrine	10.0	P. O.	1	129	42	0	-0.3	-0.5	-0.1
			4	24	7	0	0	0	0
Chlorpheniramine	2.5	P. O.	1	69	12	9	-0.8	-0.5	-0.2
			4	0	0	0	0	0	0
Chlorpheniramine	5.0	P. O.	1	124	49	12	-0.9	-0.9	-0.4
			4	0	0	0	0	0	-0.4
Chlorpheniramine	10.0	P. O.	1	204	122	19	-1.2	-1.2	-0.6
			4	23	7	0	0	0	0
Romilar [®] CF, cc./Kg	0.25	P. O.	1	44	4	9	+0.2	+0.1	0
			4	0	0	0	0	0	0
1 cc. contains: 3 mg. Romilar [®]	0.5	P. O.	1	69	32	14	-0.2	-0.1	+0.9
			4	0	0	0	0	0	0
0.25 mg. chlorphenir- amine									
1 mg. <i>l</i> -phenylephrine	1.0	P. O.	1	80	24	19	-0.4	+0.3	-1.0
			4	0	0	0	0	0	0
24 mg. N-acetyl- <i>p</i> - aminophenol	2.0	P. O.	1	114	48	19	-1.6	-0.1	-0.8
			4	7	0	0	0	0	0
	4.0	P. O.	1	176	84	29	-1.3	-0.2	-0.5
			4	41	0	0	-0.6	0	0
	8.0	P. O.	1	240	97	29	-2.6	-0.6	-1.3
			4	74	58	0	-0.9	+0.7	+0.2
Codeine	6.25	P. O.	1	58	35	0	0	0	0
			4	0	0	0	0	0	0
Codeine	12.5	P. O.	1	144	92	0	0	0	0
			4	12	0	0	0	0	0
Codeine	25.0	P. O.	1	235	136	0	0	0	0
			4	103	38	0	0	0	0
Na Salicylate	50.0	P. O.	1	40	0	14	+0.3	+0.7	+0.1
			4	0	0	0	0	0	0
Na Salicylate	100.0	P. O.	1	116	0	19	-1.5	0	0
			4	45	0	0	0	0	0
Na Salicylate	200.0	P. O.	1	209	0	24	-2.6	-0.2	+0.1
			4	132	0	10	-1.0	0	0

mental conditions employed in producing the edema in the rat's foot and relieving it with various agents, circulatory effects could exert important changes which might be measured as apparent antipyretic, antiedematous, and analgesic effects. This question has not been adequately resolved.

REFERENCES

- (1) Cass, I. J., and Frederik, W. S. *New Engl. J. Med.*, 249 (1951).
- (2) Pellmont, B., and Bachtold, H., *Schmerz und Hochsch.*, 84, 1368 (1951).
- (3) Benson, W. M., Stefko, P. L., and Randall, L. O., *J. Pharmacol. Exptl. Therap.*, 109, 189 (1954).
- (4) Isbell, H., and Fraser, H. P., *ibid.*, 107, 521 (1953).
- (5) Flinn, T. B., and Brodie, B. B., *ibid.*, 94, 76 (1948).
- (6) Boreus, L., and Sandberg, F., *Acta Physiol. Scand.*, 28, 266 (1953).
- (7) Wallenstein, S. J., and Houde, R. W., *Federation Proc.*, 13, 411 (1951).
- (8) Randall, L. O., and Schlitt, J. J., *Arch. intern. Pharmacodyn.*, 111, 409 (1957).
- (9) Schlitt, J. J., and Randall, L. O., *Federation Proc.*, 13, 103 (1951).

Paper Chromatography of I^{131} -Labeled Iodopropylidene Glycerol With Autoradiographic Identification*

By GEORGE F. HOFFNAGLE†, CATHERINE N. SIDERI,
and ARTHUR OŠOL

The paper chromatographic characteristics of iodopropylidene glycerol in various solvent systems were investigated. Certain quantitative evaluations of the chromatographic data were made possible through tagging of the compound with I^{131} . Autoradiograms of chromatograms were also prepared, in connection with which the relationship of radioactivity of test specimens to duration of exposure in preparing the autoradiograms was studied.

THIS STUDY WAS OCCASIONED by the need for developing suitable analytical techniques to be used in certain phases of an investigation of the biological distribution and fate of iodopropylidene glycerol (1, 2), hereafter referred to as IPG, a safe and orally efficacious source¹ of iodine for nutritional and therapeutic use in humans (3-6).

Specifically, knowledge of the chromatographic characteristics of IPG in various solvent systems was required in order to permit identification of the I^{131} -labeled compound(s) in the microgram quantities present in preparations of biological material (1). Location of the "spots" obtained by paper chromatography of tagged IPG was effected by scanning the paper with a Geiger-Mueller tube and attached counter. More exact delineation of the spots was achieved by preparing on X-ray film autoradiograms of the chromatograms.

EXPERIMENTAL

Chromatographic Studies.—An ascending technique, along lines described by Williams and Kirby (7), was chosen for this work. The IPG, tagged with I^{131} by an exchange method (2), was applied to the paper as a solution in chloroform. The amount of IPG applied to the paper was determined from counts of total radioactivity obtained with the scanning equipment before the chromatography was begun. Sheets of Whatman No. 1 paper were used. Applications of the solution were made at points 5 cm. apart along a line parallel to the bottom edge of the paper and 1 cm. above the solvent level. The paper was made into a cylinder by securing the vertical edges with paper clips. The chromatography chamber was saturated with the vapor of the

solvent through the night preceding the start of each chromatographic experiment.

The following solvent systems were investigated: (1) The upper phase of an equilibrium mixture of *n*-butanol and 2 *N* formic acid in water. (2) The upper phase of an equilibrium mixture of 20% *p*-dioxane in *n*-butanol with a 2 *N* aqueous solution of ammonia. (3) The upper phase of an equilibrium mixture of *n*-butanol with 0.2 *M* sodium phosphate buffer at pH 7.2. (4) Isopropyl ether saturated with water. (5) Toluene saturated with water. (6) The ethyl acetate phase of a 5:1 equilibrium mixture of ethyl acetate and water. (7) The lower phase of an equilibrium mixture of 11 volumes of carbon tetrachloride, 2 volumes of *p*-dioxane, and 1 volume of water. (8) The upper phase of an equilibrium mixture of 10 volumes of toluene, 5 volumes of isopropyl ether, and 1 volume of water. (9) Water alone. (10) Water saturated with *n*-butanol. (11) A 2-phase system composed of an immobile phase of 2% glacial acetic acid in propylene glycol, and a mobile phase of a 1:1 mixture of benzene and cyclohexane. (12) A 2-phase system composed of formamide (applied as a 30% solution in acetone) as the immobile phase, with a mixture of 20 volumes of isooctane, 10 volumes of benzene, and 1 volume of formamide as the mobile phase.

The butanol-phosphate buffer system, represented by (3) above, was chosen for the biological studies because the IPG isomers did not appreciably separate, but were sufficiently well separated from other expected iodine compounds, and the pH of the buffer prevented decomposition of iodides to free iodine.

Detection and Quantitative Evaluation of Radioactive Spots.—The completed chromatograms were air-dried, then scanned for location of radioactive spots using an end-window, organic-vapor-quenched, Geiger-Mueller counter tube enclosed in a 1.25 cm. lead shield, inverted under a 50 × 50 cm. Plexiglas table of 0.65 cm. thickness (Fig. 1). A 2 × 2 cm. opening in the table and in the lead shield over the window of the counter tube permitted counting over an area of 4 sq. cm.; light entering through the Plexiglas outlined the area of the paper being counted.

By ruling off at right angles distances of 2 cm. from the point of application of the material which had been chromatographed, the path of the migrating materials could be accurately surveyed. The initial counts of radioactivity were for one minute. The rulings permitted easy and reproducible return to any area of the paper to obtain replicate and

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† Present address: 86 Forest Ave., Verona, N. J.
Preparations of IPG used in this study were supplied by Henry K. Wampole & Co., Inc., Philadelphia, Pa.

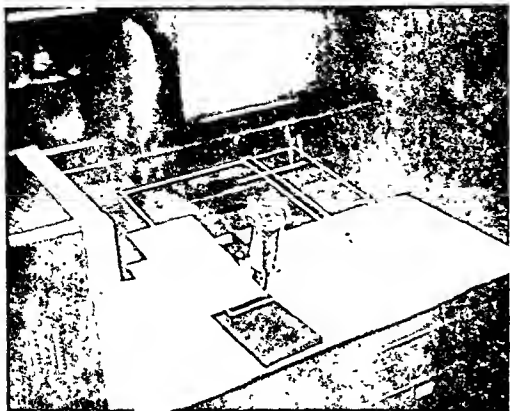


Fig. 1.—Scanning table for detection and estimation of radioactive substances on paper chromatograms.

longer counts. An estimate of relative concentrations of radioactivity of different areas on a chromatogram was obtained by subtracting the background (usually about 30 counts per minute) from the counting rate for each area, then dividing the value for each area by the sum of the values for all areas.

Autoradiographic Studies.—To facilitate location and delineation of radioactive spots, autoradiograms of the chromatograms were prepared. The film used was Kodak type K X-ray film, in $1\frac{7}{8} \times 16$ inch strips. Boyd (8) found that for low intensity exposures this film is more sensitive to I^{131} than No-Screen and other commonly available X-ray films. The film was placed flat directly over the chromatogram along the line of travel of the sample and stapled on. The origin and solvent front lines were marked on the film with India ink at the time of placement.

It was desired to continue the autoradiographic exposure long enough to record weakly radioactive spots, which procedure resulted in overexposure of strongly radioactive spots. In this connection, it was considered important to correlate the observed count of a sample on the scanning table with exposure time of the film. Pele (9) recommended an exposure time corresponding to 10^7 disintegrations per sq. cm. A series of dilutions of a sodium radio-iodide solution standardized by Abbott

Laboratories, diluted in 1% potassium iodide solution, was prepared and the corrected counts per minute obtained on the scanning table. By this method a ratio of 283 ± 4.8 counts per minute per millimicrocurie was established over the range of 36 to 1125 counts per minute, or 0.13 to 4 millimicrocuries.

A series of identical radioactive spots was prepared on Whatman No. 1 paper, having a corrected count of 275 counts per minute (0.97 millimicrocurie). After drying, a square of Kodak type K X-ray film was placed directly over each spot. The films were removed at various times as indicated in Fig. 2. All the squares were developed at the same time, in strict accordance with directions for the film. It will be noted that at 10^7 disintegrations, approximately maximum density of the image was obtained, and adequate density for detection was obtained with as little as 2.5×10^5 disintegrations.

RESULTS AND DISCUSSION

The chromatographic data obtained for IPG in different solvent systems, expressed in terms of the

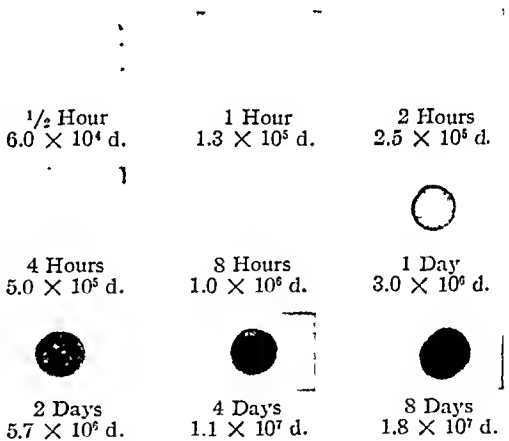


Fig. 2.—Effect of varying exposure time to identical I^{131} spots, in terms of reaction density of Kodak Type K film.

TABLE I.—CHROMATOGRAPHIC DATA FOR IPG IN VARIOUS SOLVENT SYSTEMS

Solvent	Spot 1		Spot 2		Spot 3	
	Rf Value	Component, %	Rf Value	Component, %	Rf Value	Component, %
1	0.79	6	0.85	39	0.89	55
2	0.61	92 ^a	0.90	5	0.93	3
3	0.80	7	0.84	32	0.89	61
4	0.66	6	0.83	50 ^a	0.93	44
5	0.00	79	0.97	21 ^a
6	0.96	100 ^a
7	0.00	16	0.91	24	0.97	60
8	0.96	100 ^a
9	0.82	100 ^a
10	0.79	55	0.84	45
11	0.03	42	0.89	58 ^a
12	0.00	75	0.75	25 ^a

^a Noticeable tail on autoradiogram and scanning

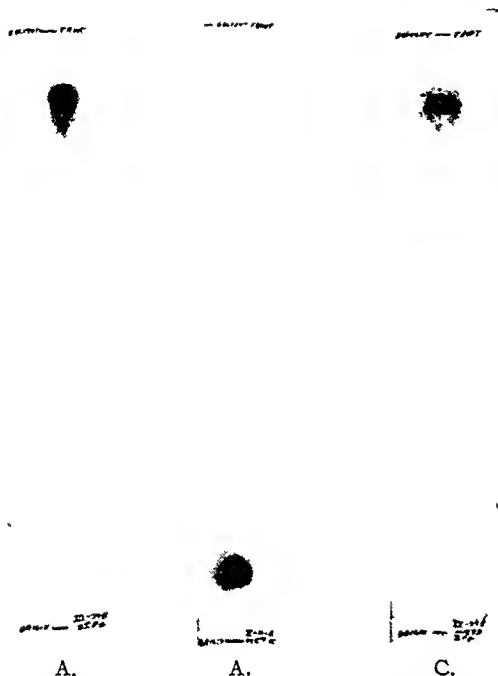


Fig. 3—Autoradiograms of chromatograms using solvent (3), *n*-butanol saturated with sodium phosphate buffer at pH 7.2. A. 21PG. B. Potassium iodide tagged with radioactive sodium iodide C. Mixed IPG.

R_f value for the spot or spots observed, with an estimate of the proportion of the component producing each spot, are given in Table I. Examples of autoradiograms of chromatograms obtained with solvent No. 3 are shown in Fig 3.

Duplicate chromatograms were run using each solvent, and in those cases where more than one spot was obtained another pair of duplicates were obtained. The appearance of multiple spots is not surprising in view of the fact that two structural isomers of IPG, differing in the position of the iodine atom in the molecule, are present, and that a theoretical total of 12 optical stereoisomers may be present, which latter, however, have not been characterized (2).

The R_f values obtained with the various solvents indicate relatively good solubility of IPG in polar solvents, including chlorinated solvents as well as water, and the relatively poor solubility of a large proportion of the preparation in hydrocarbons.

REFERENCES

- (1) Hoffnagle, G. F., and Osol, A., *THIS JOURNAL*, 47, 149 (1958).
- (2) Hoffnagle, G. F., and Osol, A., *ibid*, 47, 303 (1958).
- (3) McKnight, R. B., *A Carolina Med J*, 7, 590 (1946).
- (4) McKnight, R. B., *Southern Surgery*, 13, 810 (1947).
- (5) Ficarra, B. J., *Medical Times*, Oct. 1954.
- (6) Slaughter, D., *et al*, *Am Therap Soc Meeting*, Boston, April 13-16, 1950.
- (7) Williams, R. J., and Kirby, H. M., *Science*, 107, 481 (1948).
- (8) Boyd, G. A., "Autoradiography in Biology and Medicine," Academic Press, New York City, 1955, p. 51.
- (9) Pelc, S. R., "Radiation Dose in Autoradiography," in "Ciba Foundation Conference on Isotopes in Biochemistry," The Blackiston Company, 1951.

Development of a Germicidal Soap Containing Bithionol*

By S. H. HOPPER† and K. M. WOOD‡

The use of one per cent Tween 80 in culture media reduces the inhibitory action of hexachlorophene in surgical scrub soap tests and indicates that hexachlorophene provides no appreciable reduction in bacterial count. A mixture of surface-active agents containing three per cent bithionol gave a marked reduction in bacterial count when used under identical conditions.

PREVIOUS REPORTS have described work from our laboratory on the development of a relatively inexpensive surgical scrub soap (1). The germicide in which we were interested was bithionol¹ in the amount of three per cent by volume. Using a coconut fatty acid base the reduction in bacterial count was of the same order of magnitude as a commercial preparation con-

taining hexachlorophene. However, due to the relatively high number of personnel who developed a dermatitis on continued use of this product, it was discontinued and a search was made for a basic material which would not cause any skin reactions. This report describes the action of bithionol in a mixture of surface-active agents known as LD-44.² Little or no germicidal action would be expected of this anionic mixture in the absence of a specific germicidal agent.

EXPERIMENTAL

Methods.—The procedure was the serial basin

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¹ Monsanto Chemical Co., brand name Actamer for 2,2'-thiobis (4,6 dichlorophenol).

² Obtained from Stepan Chemical Company, Chicago, Ill.

technique as reported above for the coconut fatty acid soap. However, the culture media in this case contained 1% Tween 80³ as the inactivating agent for the germicide. Lawrence and Erlandson, (2, 3) had shown that the inhibiting action of hexachlorophene and bithionol was nullified by Tween 80 and that plate counts made with this nonionic surface-active agent in the culture media had a considerably higher number of surviving organisms than those in which serum was used as the inactivating agent.

Patch Testing.—The prophetic patch test was done according to the description given by Schwartz (4). One hundred student nurses volunteered for this purpose. The LD-44 containing bithionol did not cause any reaction, but the adhesive tape caused quite a bit. In a group of 100, the rate of positive reactions should not exceed approximately 3% according to the statistical analysis of Henderson and Riley (5). Thus the absence of any reactions in the group tested exceeds the allowable limits quoted by these authors.

Manufacture of the Germicidal Detergent.—This is given in outline as follows.

1. Materials required: liquid detergent-44 (LD-44) obtained from Stepan, sodium hydroxide pellets obtained from Mallinckrodt, bithionol obtained from Monsanto, and perfume oil compound No. 11420 obtained from Fritzsche Brothers.

2. Equipment required: mixing tanks, stainless steel, 30-gallon capacity. Agitator with variable speed, i. e., Lightening Mixer, model CV-4.

3. Formula (10 gallons): 1140.0 Gm. Actamer, 378.0 Gm. sodium hydroxide, 19000.0 cc. LD-44, 50.0 cc. perfume oil No. 11420, and distilled water to make 38000.0 cc.

4. Procedure: (a) Dissolve the sodium hydroxide in one gallon of distilled water in the pyrex bottle, then slowly add the Actamer to the hydroxide solution with constant agitation until all of the Actamer is dissolved. (b) Place the LD-44 in the stainless steel mixing tank and add the Actamer solution; mix thoroughly at low speed to avoid excessive foam formation. (c) Add the perfume and mix well, then add the remainder of the distilled water. (d) Bottle in amber containers.

RESULTS AND DISCUSSION

It is apparent from Fig. 1 that the preoperative scrub preparation containing 3% hexachlorophene did not reduce the bacterial count to any appreciable extent after eight days of continuous use. Fig. 2 shows a marked reduction in bacterial count after the LD-44 containing 3 per cent Bithionol had been used for eight days. Note that although neither preparation reduced the bacterial count to zero, the bithionol germicide reduced the bacterial flora far more effectively than the presently highly publicized hexachlorophene products. Thus the number of potentially dangerous bacteria left on the skin is also reduced. It is obvious that experimental design is tremendously important with regard to the type of results obtained; and it is therefore imperative to re-evaluate hexachlorophene in the light of the action of Tween 80 as an inhibitory agent in culture media.

It is probably true that it is not possible to sterilize the skin without destroying it. As Price (6) says, "as far as I can determine, single periods of

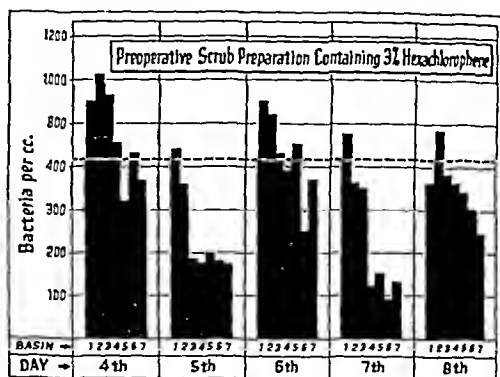


Figure 1.

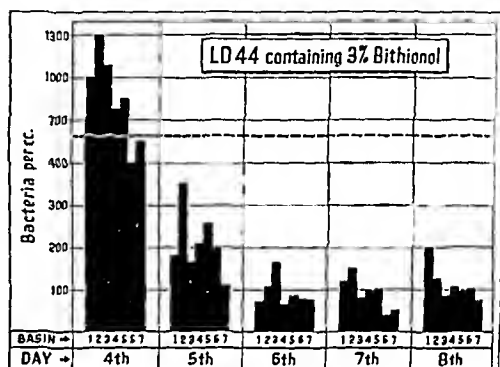


Figure 2.

washing or scrubbing from one to ten minutes with preparations containing G-11 in bar soap, liquid soap or phisoderin do not immediately reduce the cutaneous bacterial flora any more rapidly than if the washing had been done with Ivory soap."

From these observations it may be seen that a non-toxic, effective, and inexpensive germicidal scrub soap has been developed.

SUMMARY

One per cent Tween 80 in culture media reduces the inhibitory action of hexachlorophene in a surgical scrub soap and shows that there is no appreciable reduction in cutaneous bacterial count after eight days of continuous use. A mixture of surface-active agents known as LD-44 to which three per cent bithionol was added was tested under the same conditions. It did not cause any dermatitis, gave a marked reduction in bacterial count after eight days of continuous use, and reduced the bacterial flora on the hands to a considerably lower level than that obtained with the hexachlorophene preparation.

REFERENCES

- (1) Hopper, S. H., Beck, A. V. R., and Wood, K. M., *Bull. Am. Soc. Hosp. Pharmacists*, 10, 199(1953).
- (2) Lawrence, C. A., and Erlandson, A. L. Jr., *THIS JOURNAL*, 42, 352(1953).
- (3) Erlandson, A. L., Jr., and Lawrence, C. A., *Science*, 118, 274(1953).
- (4) Schwartz, L., Turlip, L., and Peck, S. M., "Occupational Diseases of the Skin", 2nd ed., Lea and Febiger, Philadelphia, 1947, pp. 306-309.
- (5) Henderson, C. R., and Riley, E. C., *J. Invest. Dermatol.*, 6, 277(1951).
- (6) Price, P. B., *Drug Standards*, 19, 161(1951).

³ Atlas Powder Company, Wilmington, Delaware, brand of polyoxyalkylene derivative of sorbitan monooleate.

The Synthesis of Some Substituted Thianaphthene-2-carboxamides and Their Antifungal Properties*

By ROBERT W. GOETTSCH† and GAIL A. WIESE

Thianaphthene-2-carboxylic acid, thianaphthene-2-carboxamide and nineteen substituted thianaphthene-2-carboxamides were prepared. All of the compounds were screened for their antifungal activity against *Trichophyton rubrum* and those compounds which appeared to be equal to or more active than undecylenic acid were tested against three other pathogenic fungi. The *in vitro* activity of the compounds was compared to the activity of three known antifungal agents: undecylenic acid, salicylanilide, and 5-chloro-8-hydroxyquinoline. Of all of the compounds tested, N-(2-methylpiperidyl)-thianaphthene-2-carboxamide, N-cyclohexylthianaphthene-2-carboxamide, and N-morpholinylthianaphthene-2-carboxamide were the most active against all four of the fungi.

THE PREPARATION of 3-substituted thianaphthene derivatives has undergone extensive study but only limited research has been directed toward the synthesis of the 2-substituted derivatives. This apparent lack of active research in the preparation of the 2-substituted derivatives of thianaphthene has been due to the lack of known procedures, other than metalation reactions, by which thianaphthene can be directly substituted on the 2-position.

In this investigation, thianaphthene-2-carboxylic acid, thianaphthene-2-carboxamide and nineteen substituted thianaphthene-2-carboxamides were prepared and tested for their antifungal activity. Thianaphthene-2-carboxylic acid has been prepared by several investigators by a metalation reaction followed by carbonation and acidification. Weissgerber and Kruber (1) found that the sodio derivatives prepared by the action of sodamide on thianaphthene in hot xylene yielded a mixture of thianaphthene-2-carboxylic acid and the 2,3-dicarboxylic acid. These investigators also reported the synthesis of the 2-carboxylic acid by the reaction of thianaphthene with sodamide in liquid ammonia. Schönberg, Petersen, and Kaltschnitt (2) reported the synthesis of thianaphthene-2-carboxylic acid by reacting thianaphthene with powdered sodium in anhydrous ether, followed by carbonation. Mayer, *et al.* (3), described the synthesis of thianaphthene-2-carboxylic acid from *o*-mercaptobenzaldehyde and chloroacetic acid. In 1950 Shirley and Cameron (4) described the synthesis of the 2-carboxylic acid by metalation

of thianaphthene with *n*-butyllithium to form 2-thianaphthenyllithium which was then converted into the desired acid by carbonation and acidification.

The thianaphthene-2-carboxylic acid used in this investigation was prepared by a modification of the method of Shirley and Cameron (4). These modifications included conducting the reaction under an atmosphere of nitrogen, and controlling the temperature from -20° to 0° for the formation of *n*-butyllithium and from 0° to 20° for the reaction of *n*-butyllithium with thianaphthene.

EXPERIMENTAL PART I

Thianaphthene-2-carboxylic acid.—A chilled, ethereal solution of 48.04 Gm. (0.75 mole) of *n*-butyllithium, prepared according to the procedure of Gilman, *et al.* (5), was placed in a three-necked flask fitted with a mercury stirrer, dropping funnel, nitrogen inlet, and a low temperature thermometer. Sixty-seven Gm. (0.5 mole) of thianaphthene¹ was dissolved in 100 ml. of anhydrous ether and added dropwise from the dropping funnel to the chilled *n*-butyllithium solution. After addition of the thianaphthene solution, the mixture was stirred for two more hours. The internal temperature of the reaction mixture was maintained at 0° to 20° with a freezing mixture of solid carbon dioxide and acetone. The ethereal solution of 2-thianaphthenyllithium was then poured over a slurry of solid carbon dioxide and anhydrous ether. After the carbon dioxide had evaporated, the mixture was diluted with 1,000 ml. of water. The aqueous layer was separated and made acid to Congo Red with concentrated hydrochloric acid. The mixture was allowed to stand overnight at room temperature to insure sufficient coagulation of the acid to permit filtration. The precipitated acid was then separated by filtration, dried and recrystallized from dilute methanol. The air-dried acid yielded 5. Gm. or 62% of the theoretical. M. p. 236° .

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¹ The thianaphthene used in this investigation was supplied through the courtesy of the Texas Company, Beaconsfield, N. Y.

Substituted thianaphthene - 2 - carboxamides—The general reaction procedure can be illustrated by the synthesis of *N* benzylthianaphthene-2-carboxamide. In a 500 ml three necked flask, fitted with a mechanical stirrer, dropping funnel, and reflux condenser, was placed 8.9 Gm (0.05 mole) of thianaphthene 2 carboxylic acid. Forty-one Gm (0.34 mole) of thionyl chloride was added dropwise and the mixture refluxed at 80° for one hour. The excess thionyl chloride was removed by distillation under reduced pressure. The resultant acid chloride was dissolved in 15 ml of anhydrous pyridine, then 10.7 Gm (0.1 mole) of benzylamine, dissolved in 15 ml of anhydrous pyridine, was added dropwise at room temperature. The reaction mixture was refluxed for three hours and then poured into 300 ml of water. The precipitated amide was separated by filtration, dried and recrystallized from a mixture of equal parts of acetone and ethyl alcohol. The purified amide was then dried in vacuum at 70°. The yield was 11.4 Gm or 85% of the theoretical. *M p* 142–143° *Anal Calcd.* N, 5.26%, Found N, 5.15%.

The conditions of the reactions for the preparation of the compounds in this investigation are given in Table I. Method A involved the addition of the amine at room temperature while in method B the reaction vessel was cooled by an ice salt freezing mixture during the addition of the amine.

EXPERIMENTAL PART II

The compounds prepared in this investigation were screened for their antifungal activity by a modification of the paper disk technique similar to that reported by Collins and Wiese (6). The fungi used were *Microsporum gypseum*, *Microsporum canis*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. Sabouraud's dextrose agar, pH 5.6, was

used as the culture medium. Aqueous suspensions of spores of the test organisms were prepared from fourteen day agar slant cultures which had been incubated at room temperature. The suspensions were prepared by adding 20 ml of sterile water for injection to the agar slant, grinding the colony growth with the aid of a sterile glass stirring rod, and transferring the suspension to a wide mouth glass bottle containing small glass beads. The colony growth was pulverized into a homogeneous suspension by agitation of the glass container. The culture plates were prepared by pouring 20 ml of the sterile culture medium into culture plates which were previously sterilized by heating in an oven at 140° for four hours. After solidification of the agar, the plates were inoculated with 1 ml of the aqueous spore suspension.

The testing procedure consisted of immersing the paper disks into an acetone solution of the compound for thirty seconds and then draining off the excess liquid by touching the disk to the walls of the container. The disks were placed on each culture plate and duplicate plates were run for each compound. The plates were then incubated at room temperature for fourteen days and the zones of inhibition recorded. The zones of inhibition were measured as the minimum distance between the periphery of the paper disk and the colony growth. Control plates containing the medium and acetone only were included in each of the tests. The *in vitro* activity of the compounds was compared to the activity of three known antifungal agents: undecylenic acid, saleylanilide, and 5 chloro-8 hydroxyquinoline.

A preliminary screening of all of the compounds was conducted against *Trichophyton rubrum* and the results of this initial testing are recorded in Table II. Only those compounds which appeared to be equal to or more active than undecylenic acid were tested against the other fungi. The results of these experiments are given in Table III.

TABLE I—SUBSTITUTED THIANAPHTHENE-2 CARBOXAMIDES

No	Amine Component	Method	Period of Re fluxing Hr	Recrystallization Solvent	Yield %	<i>M p</i>	N Caled	N, Found
1	<i>p</i> Chloroaniline	A	3	Acetone-ethanol	89	208–209	4.87	4.88
2	<i>m</i> Chloroaniline	A	3	Ethanol	54	165–166	4.87	4.83
3	<i>o</i> Chloroaniline	A	3	Ethanol	62	131	4.87	4.81
4	2,4-Dichloroaniline	A	3	Acetone ethanol	86	145	4.35	4.38
5	2,5 Dichloroaniline	A	3	Acetone ethanol	90	135–136	4.35	1.29
6	Morpholine	B	3	Ethanol	47	99–99.5	5.66	5.53
7	Benzylamine	A	3	Acetone ethanol	85	142–113	5.26	5.15
8	Cyclohexylamine	B	3	Acetone ethanol	79	161–162	5.40	5.38
9	Allyl amine	B	3	Ethanol	82	118	6.44	6.37
10	<i>p</i> -Hydroxy aniline	A	6	Acetone ethanol	66	229–230	5.20	5.23
11	2 Methylpiperidine	B	3	Ethanol	70	83	5.40	5.32
12	Phenylethylamine	A	3	Ethanol	76	123	4.97	1.91
13	<i>n</i> Hexylamine	B	3	Ethanol	86	58–59	5.35	5.26
14	<i>m</i> Toluidine	A	4	Acetone ethanol	78	156	5.21	5.10
15	<i>o</i> Anisidine	A	4	Ethanol	78	121.5–122	4.94	4.87
16	<i>p</i> Anisidine	A	4	Ethanol	77	186	4.91	1.88
17	<i>p</i> -Toluidine	A	4	Ethanol	71	165–166	5.21	5.01
18	Piperidine	B	3	Ethanol	85	125	5.70	5.53
19	2 Amino piperidine	A	3	Ethanol	78	133–133.5	11.02	11.15
20	Ammonia	B	1	Ethanol	86	176	7.90	7.86
21	Aniline	A	2	Ethanol	62	188–189	5.53	5.49

TABLE II.—ZONES OF INHIBITION IN MILLIMETERS OF THE RADII SEGMENTS AGAINST *T. RUBRUM*

Amine Component	0.25%	0.5%	1.0%	2.0%	4.0%
<i>p</i> -Chloroaniline	3	3	3.5	4	5
<i>m</i> -Chloroaniline	1	1	1	1	1
<i>o</i> -Chloroaniline	2	2	2	2	2
2,4-Dichloroaniline	0	0	0	0	0
2,5-Dichloroaniline	2	2	2	2	2
Morpholine	2	3	5	7	9
Benzylamine	2	2	2	2	2
Cyclohexylamine	12	12	12	12.5	15
Allyl amine	4	4.5	6	6	9
<i>p</i> -Hydroxyaniline	0	0	0	0	0
2-Methylpiperidine	4	7	7	7	7
Phenylethylamine	7	7.5	7.5	9.5	10
<i>n</i> -Hexylamine	5	6	7.5	8	9
<i>m</i> -Toluidine	5	5	6	6	7
<i>o</i> -Anisidine	0	0	0	0	0
<i>p</i> -Anisidine	1.5	2	3	5	6
<i>p</i> -Toluidine	5	5	6	6	6
Piperidine	0	0	0	0	0
2-Aminopyridine	3	3.5	4	4	4
Ammonia	1.5	2.5	3	6	6
Aniline	0	0	0	0	0
Thianaphthene-2-carboxylic acid	0	0	0	1	1
Undecylenic acid	0	0	2.5	4.5	4.5
5-Chloro-8-hydroxyquinoline	18	20	31	31	34
Salicylanilide	2.5	3.5	4	4	4
Acetone	0	0	0	0	0

TABLE III.—ZONES OF INHIBITION IN MILLIMETERS OF THE RADII SEGMENTS

Amine Component	%, w/v	<i>M. gypseum</i>	<i>M. canis</i>	<i>T. rubrum</i>	<i>T. mentagrophytes</i>
<i>p</i> -Chloroaniline	0.25	0	0	3	0
	0.5	0	0	3	0
	1.0	0	0	3.5	0
	2.0	0	0	4	0
	4.0	0	0	5	0
Morpholine	0.25	0	2.5	2	0
	0.5	0	3	3	0
	1.0	0	3	5	2
	2.0	2	4	7	5
	4.0	6	5	9	7
Cyclohexylamine	0.25	1	3	12	1
	0.5	1	5	12	2
	1.0	1	6	12	3
	2.0	1	6	12.5	3
	4.0	2	7	15	4
Allyl amine	0.25	0	2	4	0
	0.5	0	3	4.5	0
	1.0	0	3.5	6	0
	2.0	0	3.5	6	0
	4.0	1	4	9	0
2-Methylpiperidine	0.25	4	6	4	4.5
	0.5	4	7	7	7
	1.0	4	7	7	7
	2.0	4	8	7	7.5
	4.0	5	8	7	8
Phenylethylamine	0.25	1	2.5	7	2.5
	0.5	1	3.5	7.5	2.5
	1.0	1	6	7.5	2.5
	2.0	1	6	9.5	2.5
	4.0	1	7	10	3
<i>n</i> -Hexylamine	0.25	1	2	5	1
	0.5	1	2	6	2
	1.0	1	2.5	7.5	2
	2.0	1	2.5	8	3.5
	4.0	2	3	9	4
<i>m</i> -Toluidine	0.25	0	1	5	0
	0.5	0	1	5	0
	1.0	0	1	6	0
	2.0	0	1	6	0
	4.0	0	2	7	0

Amine Component	Cc, w/v	M. <i>gypseum</i>	M. <i>canis</i>	T. <i>rubrum</i>	T. <i>menta</i> <i>grophytts</i>
<i>p</i> -Anisidine	0.25	0	0	1.5	0
	0.5	0	0	2	0
	1.0	0	0	3	0
	2.0	0	1	5	0
	4.0	0	1	6	0
<i>p</i> -Toluidine	0.25	0	1	5	0
	0.5	0	1	5	0
	1.0	0	1	6	0
	2.0	0	1	6	0
	4.0	0	2	6	0
2-Aminopyridine	0.25	0	0.5	3	0
	0.5	0	1	3.5	0
	1.0	0	1	4	0
	2.0	0	2	4	0
	4.0	0	2	6	0
Ammonia	0.25	0	0	1.5	0
	0.5	0	0	2.5	0.5
	1.0	0	0	3	1
	2.0	0	1	6	3
	4.0	2	2	6	4.5
Undecylenic acid	0.25	0	0	0	0
	0.5	0	1	0	0
	1.0	0	1	2.5	0
	2.0	0	4	4.5	0
	4.0	1	4	4.5	1
5-Chloro-8-hydroxyquinoline	0.25	3	9	18	6
	0.5	9	16	20	12
	1.0	12	22	31	18
	2.0	20	23	31	22
	4.0	24	24	34	24
Salicylanilide	0.25	0	4	2.5	2
	0.5	3.5	6	3.5	4
	1.0	5.5	7	4	4
	2.0	6	8	4	6
	4.0	7	10	4	7
Acetone	100.0	0	0	0	0

DISCUSSION

The major impediment in the synthesis of the compounds occurred in the preparation of thianaphthene-2-carboxylic acid. The critical step in the synthesis proved to be the temperature of the reaction between *n*-butyllithium and thianaphthene. It was found that maintenance of an internal temperature of 0° to 20° was essential in order to form 2-thianaphthenyllithium. If the temperature was allowed to rise to the boiling point of the ethereal solvent, an intermediate was formed which when carbonated did not yield the desired acid. The true chemical identity of the compound has not been established but on the basis of its analysis, the empirical formula is $C_{11}H_{10}OS_2$.

The preparation of the individual substituted amides involved determining the optimum conditions for each synthesis and included: 1. variation of the temperature during the addition of the amine, 2. determination of the period of refluxing required, 3. selecting the appropriate solvent for purification.

Thianaphthene-2-carboxamide, N-phenylthianaphthene-2-carboxamide, and N-(2-pyridyl)thianaphthene-2-carboxamide have been reported in the literature (4) but were included in this investigation in order to relate their antifungal properties with those of the other compounds in this study.

The results of the antifungal studies, as shown in Tables II and III, indicate that none of the com-

pounds were very active as compared to 5-chloro-8-hydroxyquinoline. However, twelve of the compounds tested were equal to or more active than either undecylenic acid or salicylanilide against *Trichophyton rubrum*.

Only those compounds which were equal to or more active than undecylenic acid against *Trichophyton rubrum* were screened against the other organisms. Seven of the compounds appeared to be equal to or more active than undecylenic acid against *Microsporum gypseum*; five of the compounds were equal to or more active than undecylenic acid against *Microsporum canis* and six of the compounds were equal to or more active than undecylenic acid against *Trichophyton mentagrophytes*. N-(2-Methylpiperidyl)-thianaphthene-2-carboxamide, N-cyclohexylthianaphthene-2-carboxamide, and N-morpholinylthianaphthene-2-carboxamide were the most active compounds against all four of the fungi tested.

REFERENCES

- (1) Weissgerber, R., and Kruber, O., *Ber.*, B-53, 1551 (1920).
- (2) Schönberg, A., Petersen, E., and Kaltschnitt, H., *Ber.*, 66, 233(1933).
- (3) Mayer, F., Mombour, A., Lassmann, W., Werner, W., Landmann, F., and Schneider, E., *Ann.*, 488, 259(1931).
- (4) Shirley, D. A., and Cameron, M. D., *J. Am. Chem. Soc.*, 72, 2785(1950).
- (5) Gilman, H., Beel, J. A., Brannen, C. G., Bullock, M. W., Dunn, G. E., and Miller, L. S., *J. Am. Chem. Soc.*, 71, 1189(1949).
- (6) Collins, A. P., and Wiese, G. A., *This Journal*, 44, 310(1953).

A Study of the Selective Antagonism by N-Allylnormorphine Against Meperidine, Methadone, and Morphine*

By THOMAS A. LYNCH and DONALD B. MEYERS

A study has been made of the selective antagonism by N-allylnormorphine against meperidine, methadone, and morphine. The degree of protection offered by 10 mg./Kg. of N-allylnormorphine against the acute toxicity of the narcotics in mice and rats has been determined. The extent of antagonism of the narcotic-induced depression of rabbit minute respiratory volume has been determined by repeated fixed doses of N-allylnormorphine. Further work on rabbit respiration has been carried out by administration of different mixed doses of narcotic and antagonist. Tests of selective antagonism against convulsive and acute toxic effects of the narcotics in frogs have been made.

THERE IS AMPLE EVIDENCE of the specific antagonistic effect of N-allylnormorphine against the pharmacological actions of morphine and the other chemically related compounds (1-7). However, selectivity of this antagonism toward the various narcotics has not been quantitatively studied. Such a consideration may be important in determining the relative effectiveness of N-allylnormorphine in conjunction with clinically used narcotics, in elucidating the mechanism of action of the narcotics, and in establishing a suitable procedure for evaluating other N-allyl derivatives. This investigation was undertaken to determine if a selectivity in antagonism exists.

EXPERIMENTAL

Protection Against Acute Toxicity of Narcotics in Mice.—In this study 590 albino mice of mixed sex weighing 15 to 25 Gm. were used. The acute toxicity of meperidine, methadone, and morphine was determined in untreated mice, and in mice pretreated with 10 mg./Kg. of N-allylnormorphine fifteen minutes prior to the injection of the narcotic. All injections were made by the subcutaneous route. The LD_{50} 's and per cent limits of error were determined according to the method of deBeer (8).

Protection Against Acute Toxicity of Narcotics in Rats.—One hundred seventy-six albino rats of mixed sex weighing 250 to 450 Gm. were used. The acute toxicity of meperidine, methadone, and morphine was determined in untreated rats, and in rats pretreated with 10 mg./Kg. of N-allylnormorphine fifteen minutes prior to the injection of the narcotic. All injections were made by the intraperitoneal route. The LD_{50} 's and per cent limits of error were determined as with the mice.

Antagonism of Respiratory Depression by Narcotics in Rabbits.—The minute respiratory volume of the unanesthetized rabbit was measured by use of a spirometer. The displacement of the movable cylinder was recorded on a smoked kymograph by a stylus attached to the cylinder. Comparison of

minute respiratory volumes was based on the average normal which was expressed as 100%. Injections were made into the marginal ear vein.

Method I.—Antagonism of narcotic-induced respiratory depression was determined by repeated fixed doses of N-allylnormorphine. After 3 to 5 normal consistent minute respiratory volumes were recorded at two minute intervals, a dose of the narcotic was given which depressed the respiration. Then single doses of N-allylnormorphine were administered at three minute intervals until the respiration returned to normal. The total dosage of antagonist required to restore the respiration to normal was expressed in a ratio relationship to the narcotic.

Method II.—The antagonism by N-allylnormorphine was shown by the administration of mixtures of the antagonist and narcotic. After the normal respiration was recorded, various mixture ratios were administered and the minute respiratory volume recorded 3 times at five minute intervals. Then double the previous dose of the same ratio was given, and the respiration recorded 3 times at five minute intervals. The averages of each of the 3 recordings were expressed in a percentage relationship to the average normal.

Effect of N-Allylnormorphine Upon Convulsions and Acute Toxicity by Narcotics in Frogs.—Frogs of the species, *Rana pipiens*, weighing 20 to 40 Gm. were administered the drugs by way of the ventral lymph sac. After induction of convulsions in frogs by a minimal dose of the narcotic, N-allylnormorphine (10 mg./Kg.) was administered.

The acute toxicity of meperidine, methadone, and morphine was determined in 120 frogs. The LD_{50} 's and per cent limits of error were determined as previously, after which, 1 LD_{50} of each narcotic was administered to groups of 24 frogs pretreated with 10 mg./Kg. of N-allylnormorphine fifteen minutes previously. The per cent mortality was noted.

RESULTS AND DISCUSSION

Antagonism of Acute Toxicity in Mice and Rats.—The acute subcutaneous toxicities of meperidine, methadone, and morphine in untreated mice and mice pretreated with N-allylnormorphine are given in Table I; similarly, the acute intraperitoneal toxicities in rats are given in Table II.

* Received April 16, 1956, from the College of Pharmacy, Butler University, Indianapolis, Ind.

TABLE I.—ACUTE SUBCUTANEOUS TOXICITIES OF NARCOTICS IN MICE UNTREATED AND PRETREATED WITH N-ALLYLNORMORPHINE (10 MG./KG.)^a

Compound	LD ₅₀ (Per cent Limits of Error) Untreated mg./Kg.	Pretreated mg./Kg.
Meperidine	112 (95.5–105.5)	213 (91–110)
Methadone	38 (73–137)	94 (98–102)
Morphine	390 (87–115)	470 (91–107)

^a N-Allylnormorphine was given subcutaneously.

TABLE II.—ACUTE INTRAPERITONEAL TOXICITIES OF NARCOTICS IN RATS UNTREATED AND PRETREATED WITH N-ALLYLNORMORPHINE (10 MG./KG.)^a

Compound	LD ₅₀ (Per cent Limits of Error) Untreated mg./Kg.	Pretreated mg./Kg.
Meperidine	87 (95–106)	104 (85.5–117)
Methadone	18 (79–127)	40 (90–110)
Morphine	201 (92–109)	... ^b

^a N-Allylnormorphine was given intraperitoneally.

^b LD₅₀ could not be determined due to erratic response.

The extent of selective antagonism by N-allylnormorphine was evaluated by comparing the number of micromoles of narcotic protected by 1 micromole of N-allylnormorphine as shown in Table III.

TABLE III.—PROTECTION BY N-ALLYLNORMORPHINE AGAINST THE LD₅₀ OF NARCOTICS AS EXPRESSED BY THE MOLAR RELATIONSHIP

Compound	Limits of Protection, ^a micromoles/Kg.	Mean Protected Dose, ^b micromoles/Kg.
Mice		
Meperidine	259–365	10.8
Methadone	129–204	5.8
Morphine	0–441	7.7
Rats		
Meperidine	0–139	2.4
Methadone	38–87	2.2

^a Protection by 28.8 micromoles/Kg. of N-allylnormorphine, the difference of LD₅₀'s of pretreated and untreated animals

^b Protection by 1 micromole/Kg. of N-allylnormorphine.

The limits of protection were derived from the per cent limits of error of the LD₅₀'s. The lower and upper limit of each LD₅₀ was calculated by multiplying the LD₅₀ by its lower and upper per cent limits of error. The lower limit of protection was determined by subtracting the upper limit of the LD₅₀ of the untreated animals from the lower limit of the LD₅₀ of the pretreated animals. The upper limit of protection was found by subtracting the lower limit of the LD₅₀ of the untreated animals from the upper limit of the LD₅₀ of the pretreated animals. All doses were expressed as the molar equivalents. The mean protected dose by 1 micromole/Kg. of N-allylnormorphine shows that protection is greatest for meperidine and the least for methadone in mice, while protection was only slightly greater for meperidine than methadone in rats. This relative difference in protection of the two species may be due to the difference in the ratio of the antagonist to the narcotic, since the optimum

antagonistic effect may depend on this ratio, as suggested by Hart and McCawley (2) using morphine.

Antagonism of Rabbit Respiratory Depression.—Repeated Fixed Dose Antagonism.—Table IV presents the results of antagonism by repeated fixed doses of N-allylnormorphine against methadone and morphine. The average total dosage of N-allylnormorphine was expressed in a ratio relationship to the respiratory depressant dose of the narcotic. Quantitative antagonism by meperidine could not be determined by this method, since the respiratory depression produced was comparatively less pronounced and more transient than by either methadone or morphine. Morphine at a dose of 4 mg./Kg. produced an average of 52.6% depression of the normal respiration, which is approximately the same as that produced by 0.75 mg./Kg. of methadone.

TABLE IV.—REPEATED FIXED DOSE ANTAGONISM OF METHADONE AND MORPHINE RESPIRATORY DEPRESSION IN RABBITS BY N-ALLYLNORMORPHINE

Compound	No. of Rabbits	Dose, mg./Kg.	Average % Depression	Dose of NAM, μg./Kg.	Ratio ^b
Methadone	2	1.5	73.8	19	1:81
	15	1.0	66.0	16	1:74
	5	0.75	54.9	19	1:41
Morphine	2	0.50	49.5	29	1:19
	13	4.0	52.6	54	1:48
	3	3.0	53.2	91	1:33

^a Average total dosage of N-allylnormorphine (NAM) necessary to restore normal minute volume.

^b Ratio of total dosage of N-allylnormorphine to narcotic.

The average ratios of N-allylnormorphine necessary to restore the respiration to normal at the respective depressant doses of narcotics are as follows: morphine, 1:48 and methadone, 1:41. Upon conversion of these ratios to their molar equivalents, they compare as follows: morphine, 1:13.9 and methadone, 1:41.3. These results indicate very slight selective antagonism by N-allylnormorphine for morphine.

Antagonism by Mixtures of Narcotic and N-Allylnormorphine.—Comparison of the antagonism by simultaneous administration of narcotic and N-allylnormorphine is shown in Table V. The dose of the narcotic in each mixture was kept constant as follows: methadone (0.75 mg./Kg.); morphine (4.0 mg./Kg.); and meperidine (5.0 mg./Kg.). The subsequent second dose contained twice the amount of narcotic but was the same ratio as the first dose. The mixture ratios of antagonist to narcotic which approximated an equivalent response to near normal respiration are as follows: meperidine, 1:40; morphine, 1:10; and methadone, 1:5. Therefore, it seems that respiratory depression by meperidine is antagonized most effectively and methadone the least effectively.

The validity of comparing the meperidine response with that of methadone and morphine may seem questionable, since meperidine did not produce the equivalent respiratory depression. It was found that the mixture of the ratio of antagonist and narcotic was more important than the quantity of mixture administered, for the dose of the mixture in each case could be doubled without appreciable de-

TABLE V.—EFFECTS OF MIXTURE OF NARCOTICS AND N-ALLYLNORMORPHINE ON MINUTE RESPIRATORY VOLUME OF RABBITS

Compound	No. of Recordings	Ratio ^a	Average Respiratory Depression, % 1st dose	2nd dose ^b
Methadone	4	1:60	58.7	..
	2	1:20	38.4	64.7
	9	1:15	29.7	36.2
	7	1:10	13.1	16.8
	6	1:5	12.5	11.9
Morphine	2	1:80	62.5	63.7
	6	1:30	32.5	33.9
	6	1:20	17.9	19.4
	6	1:10	6.8	8.7
	6	1:5	14.0	26.5
Meperidine	4	1:40	6.0	9.0
	6	1:20	0.4	13.0 ^d
	1	1:15	9.0 ^d	29.0 ^d
	1	1:5	7.7	33.0 ^d

^a Ratio of N-allylnormorphine to narcotic.^b Two times the amount of narcotic as the first dose.^c Meperidine was administered alone.^d Increase per cent of normal respiration.

viation from the response of the first dose as suggested by the data in Fig. 1. The lack of conformity of the meperidine curves does not contradict the above statement, since the curves above the 100% of normal respiration are due possibly to synergism of convulsiveness rather than antagonism. The results show the same qualitative relationship of selectivity as obtained with acute toxicity in mice. These results are probably more representative of the true antagonistic effect than those obtained by the repeated dose method, for the narcotic and antagonist in this case were allowed to act simultaneously. Also, the variability of the repeated dose method was eliminated.

Relationship of N-Allylnormorphine to Narcotics in Frogs.—Convulsions.—In an effort to determine narcotic antagonism in frogs, it was found that N-allylnormorphine (10 mg./Kg.) failed to prevent convulsions induced by meperidine (250 mg./Kg.)

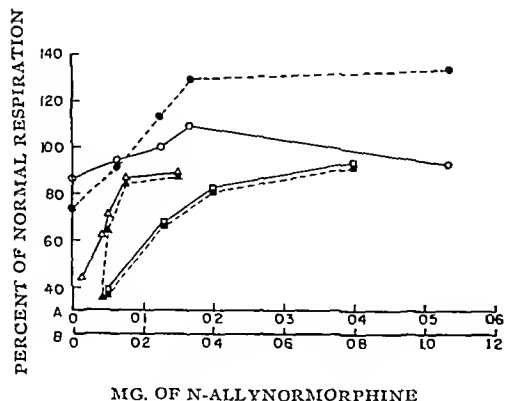


Fig. 1—Effect of different mixtures of narcotic and N-allylnormorphine upon rabbit respiratory volume.

○ Meperidine (5 mg./Kg.); △ Methadone (0.75 mg./Kg.); □ Morphine (4 mg./Kg.); ● Meperidine (10 mg./Kg.); ▲ Methadone (1.5 mg./Kg.); ■ Morphine (8 mg./Kg.); —A, - - -B.

and morphine (500 mg./Kg.), while convulsions could not be produced by methadone.

Acute Toxicity.—The acute intralymphatic toxicity of meperidine, methadone, and morphine in frogs was determined, after which, 1 LD₅₀ of these compounds were administered to frogs pretreated fifteen minutes before with N-allylnormorphine (10 mg./Kg.). As shown in Table VI, N-allylnormorphine increased the acute toxicity of methadone and meperidine in reverse order of the size of the LD₅₀. The effect upon toxicity may be attributed to synergism of N-allylnormorphine and narcotic on the spinal reflexes of the frog, in light of the fact

TABLE VI.—EFFECT OF N-ALLYLNORMORPHINE (10 MG./KG.)^a PRETREATMENT ON THE ACUTE INTRALYMPHATIC TOXICITY OF NARCOTICS IN FROGS

Compound	LD ₅₀ ^b (%) Limits of Error), mg./Kg.	No. of Frogs	% Mortality
Meperidine	191(97-104)	24	66.7
Methadone	67(97-103)	24	100.0
Morphine	590(64-150)	24	54.1

^a N-Allylnormorphine was given intralymphatically fifteen minutes prior to the narcotic.^b LD₅₀ of untreated frogs and the dose given intralymphatically to pretreated frogs.

that N-allylnormorphine is known to produce hyperactivity of the hindlimb reflexes of the chronic spinal dog, as reported by Wikler and Carter (9).

SUMMARY

1. The selective antagonism of meperidine, methadone, and morphine by N-allylnormorphine has been determined.

2. N-Allylnormorphine exhibited greatest selectivity of antagonism for meperidine and the least for methadone in protecting against acute toxicity in mice. Selective antagonism of meperidine was not significantly greater than methadone in rats.

3. Antagonism was most selective for meperidine and the least selective for methadone on rabbit respiration by the mixture method, while little selectivity was shown by the repeated dose method.

4. Antagonism by N-allylnormorphine could not be shown against the convulsions or acute toxicity in frogs; on the contrary, acute toxicity of methadone and meperidine was augmented.

REFERENCES

- (1) Hart, R. E., *Fed. Proc.*, 2, 82(1943).
- (2) Hart, R. E., and McCawley, E. L., *J. Pharmacol. Exptl. Therap.*, 82, 339(1914).
- (3) Unna, K., *ibid.*, 79, 27(1943).
- (4) Winter, C. A., Orabovats, P. D., Flataker, L., Lehman, E. G., and Lehman, J. T., *ibid.*, 111, 152(1954).
- (5) Huggins, R. A., Glass, W. G., and Bryan, A. R., *Proc. Soc. Exptl. Biol. Med.*, 75, 510(1950).
- (6) Huggins, R. A., Glass, W. G., and Bryan, A. R., *J. Pharmacol. Exptl. Therap.*, 101, 19(1951).
- (7) Radoff, L. M., and Huggins, S. E., *Proc. Soc. Exptl. Biol. Med.*, 78, 879(1952).
- (8) deBeer, E. J., *J. Pharmacol. Exptl. Therap.*, 85, 1(1945).
- (9) Wikler, A., and Carter, L. C., *ibid.*, 109, 92(1953).

A Study on Couch Grass Ergot^{*}

By LEE C. SCHRAMM† and JACK L. BEAL

Ergot found growing on couch grass in Delaware and Franklin Counties in the central part of Ohio was found to contain above N. F. X limits in ergotovine content, and below N. F. X limits in ergonovine content. It was also noted that part of the sample was infested with a small beetle-like insect. The infested portions assayed lower in alkaloid and fat content. Evidence was presented by paper chromatography to show the presence of a number of ergot alkaloids and lysergic acid.

Claviceps purpurea (Fries) Tulasne is a parasitic fungus commonly occurring on rye. Ergot of commerce is defined as the sclerotium, or resting stage, of *Claviceps purpurea*—a hard, purplish, somewhat curved body, the size of which is usually determined by the size of the grain it parasitizes. It has been noted that *Claviceps purpurea* infects other members of the Gramineae (grass family) (1) particularly the cereal grasses.

In the summer of 1956 it was noticed that there was a local infection of *Agropyron repens* (Linne) Beauvois (couch grass, quack grass, triticum) with *Claviceps purpurea*. Couch grass was noted to grow along roads and highways throughout the central part of Ohio usually along the fence bordering a wheat field. It was also noted that the wheat adjacent to the infected couch grass was infected with ergot. Couch grass, a member of the cereal grasses, is a perennial weed, the aerial portion consisting of a leafy culm attaining the height of 1 meter, and bearing a terminal spike of many spikelets (2). The rhizome of this grass, known as Triticum, has been used as a demulcent, diuretic, and was official in the U. S. P. from 1885 to 1926, and the N. F. from 1926 to 1950.

Since a survey of the literature failed to disclose any pharmacognostical studies of *Claviceps purpurea* found growing on couch grass, it was decided that an investigation should be made. This investigation includes the determination of the fat and moisture content, the determination of total alkaloid content, and a study of the individual alkaloids contained therein.

On further study of the individual sclerotia it was found that about one half of the sample was infested by a small beetle, thus, another division of the investigation was made, that is to determine what effect, if any, this infestation had on the fat, moisture, and alkaloid content.

The identity of the fungus as *Claviceps purpurea* and the grass as *Agropyron repens* was

verified by the botany department of The Ohio State University.¹

The average size of the sclerotia was found to be 10–12 mm in length (ranging from 6 to 18 mm.) and 1–2 mm in width. The size relative to rye ergot may be observed in Fig. 1.

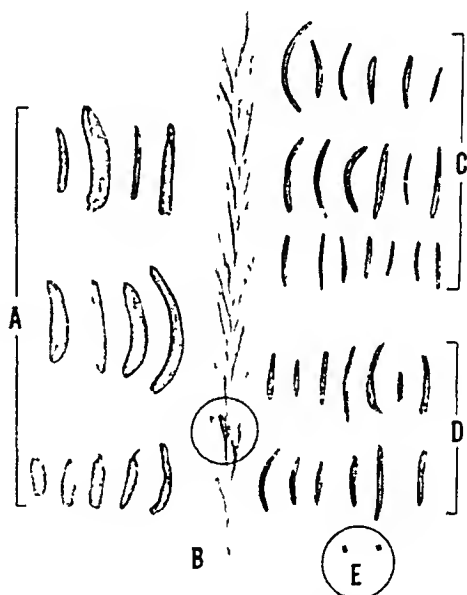


Fig. 1—(A) Rye Ergot (N.F.X.), (B) Spike of *Agropyron repens* infected with *Claviceps purpurea* (encircled), (C) Non infested couch grass ergot, (D) Infested couch grass ergot, (E) Beetles from infested couch grass ergot (encircled).

EXPERIMENTAL PROCEDURE

Samples were taken in July of 1956 from different sections of the central part of Ohio. The sclerotia were separated from the grasses, and then subdivided into two portions, the noninfested and the infested portions. The two portions were then independently passed through a Wiley Laboratory Mill and ground to a No. 20 powder.

^{*} Received September 23, 1957, from the College of Pharmacy, Ohio State University, Columbus.

† Recipient of the Kilmear Prize, 1957.

¹ The authors express their appreciation to Dr. John A. Schmitt, Department of Botany and Plant Pathology, Ohio State University, for verifying the identity of the fungus.

Moisture Determination.—The moisture determination was carried out according to the N. F. method (3). The results are listed in Table I.

Fat Determination.—Three 500-mg. samples of each portion of the ground drug were extracted by continuous extraction in Soxhlet apparatus with petroleum ether for twenty hours. The extracted matter was then transferred to a tared evaporating dish, the extraction apparatus rinsed with three small portions of the solvent, the rinsings added to the extracted matter, and the solvent evaporated to dryness over low heat. The dish was then dried in an oven at 105° to a constant weight. The amount of residue was then determined and the per cent total fat calculated from the data. The results are listed in Table I.

TABLE I.—RESULTS OF ANALYSIS

	Noninfested	Infested
Moisture, ^a %	9.2	9.2
Fat, ^a %	15.9	13.3
Alkaloids ^b		
Water-insoluble, ^c		
mg. %	198.05	177.86
Water-soluble, ^d		
mg. %	3.93	3.19

^a Average of 3 samples.

^b Average of 4 samples expressed as moisture and fat free.

^c Calculated as ergotoxine (3).

^d Calculated as ergonovine (9).

Alkaloid Determination.—The alkaloid determination was carried out according to the method of Silber and Schulze (4) with modifications. Four 200-mg. samples of both portions of the defatted material were extracted by means of continuous extraction in Soxhlet apparatus with a mixture of 0.1 ml. 10% ammonia solution and 6 ml. ether U. S. P. per 100 mg. of sample. Extraction was continued to exhaustion, which was experimentally shown to be approximately six hours. The percolate was removed, the apparatus washed with three successive 3-ml. portions of ether, placed in a 25-ml. flask, and concentrated to 5 ml. at a temperature not above 40°. This concentrated percolate was then transferred to a 30-ml. separatory funnel, and the flask rinsed with three successive 1-ml. portions of ether, the rinsings being added to the percolate. This was then shaken first with three successive 3-ml. portions of pH 8 phosphate buffer solution to extract the water-soluble alkaloids, and then with three successive 3-ml. portions of 2% tartaric acid solution to remove the water-insoluble alkaloids. Each shake-out was made up to exactly 10 ml., and a 2-ml. aliquot was taken for the assay. Each 2 ml. of unknown sample was mixed in a Fisher Electrophotometer Cell with 4 ml. of *p*-dimethylaminobenzaldehyde T. S. and immediately exposed to a mercury vapor lamp for seven minutes. The optical density was then determined in a Fisher Electrophotometer at 595 μ . For each individual determination, a blank was prepared using 2 ml. of 2% tartaric acid solution and 4 ml. of *p*-dimethylaminobenzaldehyde T. S., illuminated for the same length of time, and the meter adjusted to zero optical density.

A reference graph was prepared using U. S. P. reference standard ergonovine maleate in concentra-

tions ranging from 2 to 100 μ g. per ml. in 1:1,000 tartaric acid solution. The same procedure as was used in the assay was followed in determining the values for the reference graph. The results of the assay are listed in Table I. The illuminating lamp was of the mercury-vapor type as is supplied with the Lumetron colorimeter.

The *p*-dimethylaminobenzaldehyde reagent was prepared after the U. S. P. XV (5) and the phosphate buffer solution was prepared after Silber and Schulze (4).

Identification.—Identification of the water-insoluble fraction of the alkaloids was carried out by means of descending paper partition chromatography, using the tartaric acid solutions obtained in the assay. The solutions were made alkaline with ammonia T. S. and extracted with ether. Wide sheets of Whatman No. 1 paper (7 $\frac{1}{2}$ inch x 18 inch) were used for this experiment, since multiple separations could be run concurrently on the same paper, saving time, and eliminating variations in degree of saturation of the chamber. The papers were saturated with formamide, accomplished by passing them through a solution of acetone-formamide 3:1 and allowing the excess solvent to evaporate. The solutions of the alkaloids were applied to the starting line with a micro-pipet in 20 μ l. amounts. The starting line was about 4 inches from the end of the paper to allow the solvent front to become equable as it crossed the line (6). The mobile phase consisted of benzene saturated with formamide, and the paper was developed in a previously saturated chamber (7). Development required approximately three hours; allowing the solvent front to run $\frac{3}{4}$ to $\frac{7}{8}$ the length of the paper. The results are listed in Table II.

TABLE II.—*R_f* TABLE USING THE BENZENE-FORMAMIDE PROCEDURE FOR WATER-INSOLUBLE ALKALOIDS

Alkaloids	<i>R_f</i> Values by Kolsek (7)	Range of <i>R_f</i> Values of N. F. Ergot ^a	Range of <i>R_f</i> Values of Couch Grass Ergot ^a
Ergotamine	0.11	0.08–0.11	0.08–0.09
Ergosine	0.13	0.12–0.16	0.14–0.16
Ergotaminine	0.40	0.40–0.45	0.39–0.41
Ergosinine	0.48	0.48–0.55	0.50–0.53
Ergocornine	0.52	0.54–0.63	0.58–0.62
Ergocristine			
Ergocryptine	0.62	0.65–0.76	0.69–0.72
Ergocorninine			
Ergocristinine	0.86	0.81–0.90	0.79–0.85
Ergocryptinine			
Lysergic Acid	0.00	0.00	0.00

^a Represents 12 determinations.

Identification of the water-soluble fraction of the alkaloids was carried out by the method of Tyler and Schwarting (8) using the buffer solutions obtained in the assay. The mobile phase consisted of the upper layer of a normal butanol-glacial acetic acid-water mixture 4:1:5. The aqueous alkaloid solutions were applied to the starting line in 40 μ l. amounts to insure concentrations of the alkaloids adequate for detection. Control spots were used along with the unknown spots, consisting of reference standard ergonovine maleate in 2% tartaric

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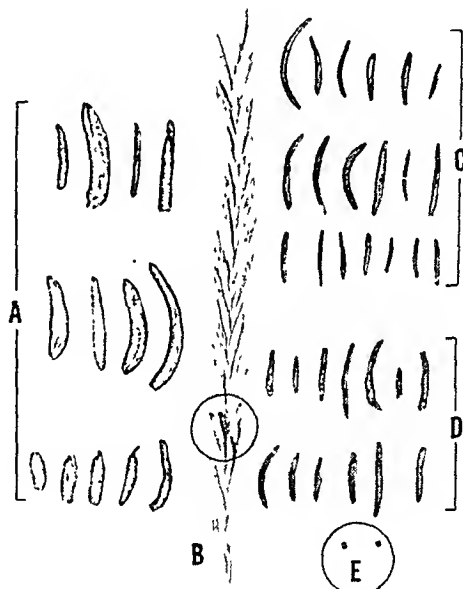


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¹ The authors express their appreciation to Dr. John A. Schmitt, Department of Botany and Plant Pathology, Ohio State University, for verifying the identity of the fungus.

Moisture Determination.—The moisture determination was carried out according to the N. F. method (3). The results are listed in Table I.

Fat Determination.—Three 500-mg. samples of each portion of the ground drug were extracted by continuous extraction in Soxhlet apparatus with petroleum ether for twenty hours. The extracted matter was then transferred to a tared evaporating dish, the extraction apparatus rinsed with three small portions of the solvent, the rinsings added to the extracted matter, and the solvent evaporated to dryness over low heat. The dish was then dried in an oven at 105° to a constant weight. The amount of residue was then determined and the per cent total fat calculated from the data. The results are listed in Table I.

TABLE I.—RESULTS OF ANALYSIS

	Noninfested	Infested
Moisture, ^a %	9.2	9.2
Fat, ^a %	15.9	13.3
Alkaloids ^b		
Water-insoluble, ^c		
mg. %	198.05	177.86
Water-soluble, ^d		
mg. %	3.93	3.19

^a Average of 3 samples.

^b Average of 4 samples expressed as moisture and fat free.

^c Calculated as ergotamine (3).

^d Calculated as ergonovine (9).

Alkaloid Determination.—The alkaloid determination was carried out according to the method of Silber and Schulze (4) with modifications. Four 200-mg. samples of both portions of the defatted material were extracted by means of continuous extraction in Soxhlet apparatus with a mixture of 0.1 ml. 10% ammonia solution and 6 ml. ether U. S. P. per 100 mg. of sample. Extraction was continued to exhaustion, which was experimentally shown to be approximately six hours. The percolate was removed, the apparatus washed with three successive 3-ml. portions of ether, placed in a 25-ml. flask, and concentrated to 5 ml. at a temperature not above 40°. This concentrated percolate was then transferred to a 30-ml. separatory funnel, and the flask rinsed with three successive 1-ml. portions of ether, the rinsings being added to the percolate. This was then shaken first with three successive 3-ml. portions of pH 8 phosphate buffer solution to extract the water-soluble alkaloids, and then with three successive 3-ml. portions of 2% tartaric acid solution to remove the water-insoluble alkaloids. Each shake-out was made up to exactly 10 ml., and a 2-ml. aliquot was taken for the assay. Each 2 ml. of unknown sample was mixed in a Fisher Electrophotometer Cell with 4 ml. of *p*-dimethylaminobenzaldehyde T. S. and immediately exposed to a mercury vapor lamp for seven minutes. The optical density was then determined in a Fisher Electrophotometer at 595μ. For each individual determination, a blank was prepared using 2 ml. of 2% tartaric acid solution and 4 ml. of *p*-dimethylaminobenzaldehyde T. S., illuminated for the same length of time, and the meter adjusted to zero optical density.

A reference graph was prepared using U. S. P. reference standard ergonovine maleate in concentra-

tions ranging from 2 to 100 μg. per ml. in 1:1,000 tartaric acid solution. The same procedure as was used in the assay was followed in determining the values for the reference graph. The results of the assay are listed in Table I. The illuminating lamp was of the mercury-vapor type as is supplied with the Lumetron colorimeter.

The *p*-dimethylaminobenzaldehyde reagent was prepared after the U. S. P. XV (5) and the phosphate buffer solution was prepared after Silber and Schulze (4).

Identification.—Identification of the water-insoluble fraction of the alkaloids was carried out by means of descending paper partition chromatography, using the tartaric acid solutions obtained in the assay. The solutions were made alkaline with ammonia T. S. and extracted with ether. Wide sheets of Whatman No. 1 paper (7½ inch x 18 inch) were used for this experiment, since multiple separations could be run concurrently on the same paper, saving time, and eliminating variations in degree of saturation of the chamber. The papers were saturated with formamide, accomplished by passing them through a solution of acetone-formamide 3:1 and allowing the excess solvent to evaporate. The solutions of the alkaloids were applied to the starting line with a micro-pipet in 20 μl. amounts. The starting line was about 4 inches from the end of the paper to allow the solvent front to become equable as it crossed the line (6). The mobile phase consisted of benzene saturated with formamide, and the paper was developed in a previously saturated chamber (7). Development required approximately three hours; allowing the solvent front to run ¾ to ⅞ the length of the paper. The results are listed in Table II.

TABLE II.—*R_f* TABLE USING THE BENZENE-FORMAMIDE PROCEDURE FOR WATER-INSOLUBLE ALKALOIDS

Alkaloids	<i>R_f</i> Values by Kolsek (7)	Range of <i>R_f</i> Values of N. F. Ergot ^a	Range of <i>R_f</i> Values of Couch Grass Ergot ^a
Ergotamine	0.11	0.08–0.11	0.08–0.09
Ergosine	0.13	0.12–0.16	0.14–0.16
Ergotaminine	0.40	0.40–0.45	0.39–0.41
Ergosinine	0.48	0.48–0.55	0.50–0.53
Ergocornine	0.52	0.54–0.63	0.58–0.62
Ergocristine			
Ergocryptine	0.62	0.65–0.76	0.69–0.72
Ergocorninine			
Ergocristinine	0.86	0.81–0.90	0.79–0.85
Ergocryptinine			
Lysergic Acid	0.00	0.00	0.00

^a Represents 12 determinations.

Identification of the water-soluble fraction of the alkaloids was carried out by the method of Tyler and Schwarting (8) using the buffer solutions obtained in the assay. The mobile phase consisted of the upper layer of a normal butanol-glacial acetic acid-water mixture 4:1:5. The aqueous alkaloid solutions were applied to the starting line in 40 μl. amounts to insure concentrations of the alkaloids adequate for detection. Control spots were used along with the unknown spots, consisting of reference standard ergonovine maleate in 2% tartaric

acid solution in a concentration of 200 μg per ml, applied in 20 μl amounts. Development time was considerably longer, requiring approximately six to twelve hours, the longer time being more desirable. The results are listed in Table III.

TABLE III— R_f TABLE USING THE *n* BUTANOL-ACETIC ACID WATER PROCEDURE FOR WATER SOLUBLE ALKALOIDS

	Range of R_f Values ^a	Average
Ergonovine reference standard	0.60–0.66	0.63
Ergonovine (Couch grass)	0.64–0.66	0.64

^a Represents 6 determinations

Since the data in Table II indicate the possible presence of lysergic acid, a further check for the presence of lysergic acid was carried out by chromatographing fresh samples of both the tartaric acid fraction and the buffer solution fraction. The procedure was the same as that used for the identification of the water soluble fraction of the alkaloids. A reference sample of lysergic acid² was chromatographed concurrently in the same chamber as that used for the unknown solutions. The results are listed in Table IV.

TABLE IV— R_f TABLE USING THE *n* BUTANOL-ACETIC ACID WATER PROCEDURE FOR CHECKING THE PRESENCE OF LYSERGIC ACID

Sample	Range of R_f Values	Average R_f^a
Lysergic acid in tartaric acid extract	0.24–0.26	0.24
Lysergic acid reference	0.23–0.27	0.25
Lysergic acid in buffer extract	0.27–0.30	0.29
Lysergic acid reference	0.30–0.33	0.32

^a Average of 6 determinations

DISCUSSION

While the moisture content was the same for the infested and noninfested portions, there was a difference in the fat content. The noninfested portion contained approximately 2.5% more fat than the infested. This could be explained on the basis of a liking by the beetle for the lipid portion of the sclerotium, or on the basis of the weight added by the contamination of the sample by the beetle.

Silber and Schulze (4) claimed that a complete extraction of alkaloids could be obtained merely by shaking the ground drug with solvent for two hours, but it was felt that Soxhlet extraction to exhaustion was certain of being complete.

The noninfested ergot showed a larger amount of water soluble and water insoluble alkaloids than the infested ergot, the difference in each case followed approximately the same ratio. In both cases the water insoluble fraction was above the N.F. limit (10) while the water soluble fraction was below the limit.

In the paper chromatography experiments for the identification of the individual alkaloids only the noninfested ergot was used.

It is well known that R_f values are modified by many factors, for example, temperature, concentration, and ratio of concentrations. Positive identification of alkaloids is impossible unless the above factors remain constant. It is often desirable to have pure samples of each alkaloid in question to use as R_f reference standards. However, in this case, this would be of little value because the exact ratio of concentrations of the alkaloids of the unknown sample cannot be ascertained. Bearing the above statements in mind, it was believed that the only possible method of identifying the alkaloids on the chromatogram in this experiment was to follow a procedure which accounted for the alkaloids commonly occurring in ergot. Such a procedure was that of J. Kolsek (7) whereby he reported R_f values of the major ergot alkaloids singly and in mixtures.

In one particular experiment, using a known mixture, he reported 7 R_f values which accounted for 10 water insoluble alkaloids of ergot. In this case, ergocornine and ergocristine were not separated, representing one spot, and ergocornine, ergocristine, and ergoeryptine were also not separated, representing one spot. As can be observed in Table II, the R_f values obtained in this experiment were similar but not identical to those of Kolsek. In addition, there was no overlapping in the ranges of R_f values. This similarity is not positive proof of identification, however, in referring to Table II there is evidence that the sequence of identity of the spots would be the same as Kolsek's. For example, in Table II, if Kolsek's 4th spot, R_f 0.48 was ergosine, the 4th spot of couch grass ergot, R_f 0.50–0.53 is also ergosine.

The identification uses nomenclature of the ergotamine group of alkaloids by Stoll as reported by Manske and Holmes (11), namely that ergotamine is not homogenous, but a complex mixture of ergocristine, ergoeryptine, and ergocornine and may contain their isomers.

The alkaloid of the water soluble fraction was identified as ergonovine, as is shown in Table III.

The possibility of the presence of lysergic acid is indicated in Table II and Table IV. Kolsek reports (7) that in using the benzene formamide procedure lysergic acid does not move, thus the R_f is zero. In the water insoluble alkaloidal fraction of couch grass ergot there was such a spot having a zero R_f as indicated in Table II. Table IV lists the R_f values for the suspected lysergic acid in the water insoluble alkaloidal fraction as well as in the water soluble fraction. In each case the R_f for the suspected lysergic acid is very close to that of a known sample of lysergic acid. This similarity of R_f in two different mobile phase systems, the benzene formamide and the *n* butanol glacial acetic acid–water mixture, gives some evidence for the presence of lysergic acid. In each case the spot indicated, by reason of its faintness, only a small amount of the substance to be present. Assuming the substance to be lysergic acid, this study does not determine whether the lysergic acid was originally present in the ergot or whether its origin is due to the extraction procedure.

Since this problem was undertaken, it was brought to our attention that Silber and Bischoff (12) have analyzed ergot of *Agropyron repens* Pal Beauvois

² The authors thank Eli Lilly and Co. for providing the sample of lysergic acid used in this experiment.

and reported total alkaloid content as ergotamine in three different samples. The values were reported as 110, 102, and 375 mg %. No other determinations were made.

SUMMARY AND CONCLUSIONS

Ergot of couch grass was studied with respect to the fat, moisture, and alkaloid contents. It was found that half of the sample was infested with a beetle, and determinations were extended to find out what effect this infestation had on the contents of the drug. It was found that:

1. The moisture content of both infested and noninfested portions was the same.
2. The fat content of infested ergot was lower than that of the noninfested portion.
3. The alkaloid content of the infested ergot was lower than that of the noninfested portion.
4. Both portions assayed above N. F. limits in ergotoxine content, and below N. F. limits in ergonovine content.

5. Evidence was presented to show that ergotamine, ergotaminine, ergosine, ergosinine, ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, and lysergic acid were probably present.

6. Ergonovine was positively shown to be present.

REFERENCES

- (1) Youngken, Heber W., "Textbook of Pharmacognosy," 6th Ed., Blakiston Co., New York, 1948, pp. 85-91.
- (2) *Ibid.*, p. 132-137.
- (3) "The National Formulary," 10th ed., Mack Publishing Co., Easton, Pa., 1955, p. 217.
- (4) Silber, A., and Schulze, T., *Pharmazie*, 8, 675 (1953).
- (5) "Pharmacopoeia of the United States," 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 1094.
- (6) Personal contact with C. Pugh, Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.
- (7) Kolsek, J., *Microchim. Acta*, 9, 1377 (1956).
- (8) Tyler, V. E., Jr., and Schwartz, A. E., *This Journal*, 41, 345 (1952).
- (9) "The National Formulary," 10th ed., Mack Publishing Co., Easton, Pa., 1955, p. 218.
- (10) *Ibid.*, p. 216.
- (11) Manske, R. H. F., and Holmes, H. L., "The Alkaloids, Chemistry and Physiology," Academic Press, Inc., New York, N. Y., 1952, p. 377.
- (12) Silber, A., and Bischoff, W., *Arch. Pharm.*, 288, 124 (1955).

Decomposition of Aspirin in the Solid State*

By LEWIS J. LEESON† and ALBERT M. MATTOCKS

The decomposition of aspirin in the solid state has been demonstrated to be dependent on vapor pressure and temperature. A possible mechanism of decomposition is offered, which consists of an initial sorption of a water layer by each particle, diffusion of aspirin into solution, and decomposition taking place by acid catalyzed hydrolysis. A series of equations which describe the mechanism have been derived and the experimental data demonstrated to fit them. Using these equations, the various kinetic constants have been determined.

SINCE SOLID DOSAGE FORMS comprise a large segment of the drugs marketed today, there is a great need for methods of predicting their stability under various conditions of storage. In order for such a method to be dependable it must be based upon a knowledge of the mechanisms involved and quantitative studies of the variables affecting the reactions.

Many pharmaceuticals are known to exhibit decomposition in solid form, and perhaps the most widely used one is aspirin. Aspirin is an especially interesting example of this type of reaction since it is known to be affected not only by temperature and humidity but also by numerous chemical agents with which it is often combined.

Decomposition of aspirin in solid form has been noted by Tsakalotos (1), Paolini (2), and

Strathopoulos (3). In addition, Ribeiro, *et al.* (4), found that certain lubricants, notably stearates, increased the degree of decomposition of aspirin contained in aspirin-phenacetin-caffeine tablets. Ebert (5), and Yamamoto and Takahashi (6, 7) studied the accelerating effects of certain amines, temperature, humidity, pressure, and grinding upon aspirin loss in tablets. These studies were concerned primarily with effects of various chemical agents and conditions and did not attempt to determine reaction rates or mechanisms.

There have been a number of kinetic studies on aspirin decomposition in solution (8-11), the most complete investigation being reported by Edwards (12). He agreed with other workers that the decomposition is first order with respect to aspirin concentration, but by studying the reaction at various pH values he demonstrated that the apparent first order rate constant is actually a composite of six rate constants. Each

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of the six constants is associated with one of the hydrolytic reactions depending upon hydrogen ion, hydroxyl ion, or water reacting with either molecular or ionic aspirin.

There is no direct method for applying to solid forms information obtained from studies of decomposition of a drug in solution, since it is even questionable whether the same reactions occur. Thus it is important that studies be made of reactions of drugs in solid state, and such a study was undertaken in these laboratories using aspirin as the first example.

EXPERIMENTAL

Aspirin powder of U. S. P. grade (Mallinckrodt) was used for this investigation. It was classified into various sizes by means of U. S. Standard sieves. Extent of aspirin decomposition was determined by measurement of the salicylic acid formed; this was accomplished by a modification of the A.O.A.C. procedure (13). The sample was dissolved in 100 ml. absolute alcohol, a suitable aliquot was transferred to a 100-ml. volumetric flask, and sufficient absolute alcohol was added to make the total alcohol content 50 ml. Two milliliters of ferric ammonium sulfate T. S. was added and the solution was made up to volume with water. The color intensity was measured on a spectrophotometer at 532 $m\mu$.

Since it was not known whether aspirin would decompose if no moisture were present, a preliminary experiment was run in the absence of water vapor. One-half gram samples of aspirin of 100/140 screen size were sealed in ampuls and stored at 35°, 45°, 60°, 80°, 100°, and 110°. Similar samples to which 2.5% calcium stearate had been added were stored under identical conditions. Calcium stearate was added since it has been reported to induce extensive decomposition of aspirin (4). Samples were removed at various times over a period of fifty days and assayed.

Samples of aspirin alone showed little or no decomposition at 80° or below. Those containing calcium stearate decomposed in a short time to the extent of about 1% and then remained constant. In samples stored at 100° and 110° salicylic acid was found to increase rapidly to about 2% and then decrease gradually with time. These samples were observed to melt and change color, and it is not known whether the color interfered with the assay or the decrease in salicylic acid might be due to salicylide formation.

It is believed that the small amount of decomposition detected in these samples could have been caused by traces of moisture which contaminated the dry aspirin during the process of filling the ampuls. The amount of water necessary to account for the decomposition observed is approximately 10^{-5} moles. It was concluded, therefore, that decomposition of aspirin in the absence of moisture is of minor importance. Consequently, attention was directed to the decomposition of aspirin in the presence of water vapor.

A series of samples of aspirin of screen size 100/140, weighing approximately 0.25 Gm., were placed in loosely capped, screw-top vials which were stored

at 50°, 60°, and 80°; and at varying humidities obtained by use of saturated inorganic salt solutions (14). At various times samples were removed and analyzed for extent of decomposition. Results are shown in Table I. A graph showing the general shape of the curve relating decomposition to time is shown in Fig. 1.

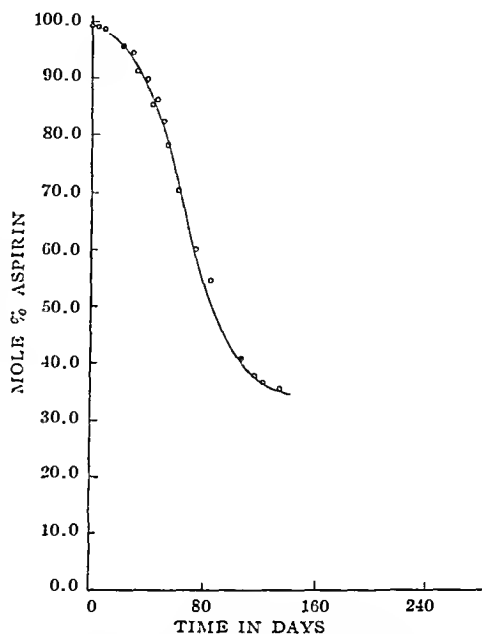


Fig. 1.—Typical decomposition of aspirin in solid state (60°, vapor pressure 120.3 mm.).

In order to be certain that the hydrolysis reaction was the only one of major importance and that no salicylic acid was lost by volatilization, an ultra-violet assay for aspirin and salicylic acid (5) was performed on numerous samples. This method consisted of reading solutions of aspirin and salicylic acid in absolute alcohol at 226 $m\mu$ and 235 $m\mu$, which are the absorbance peaks for aspirin and salicylic acid, respectively. The amount of each present was then determined by the usual two-component system technique. In all cases the original amount of aspirin could be accounted for by a summation of the aspirin and salicylic acid found.

TREATMENT OF THE DATA

Examination of the data of Table I by means of typical plots used to determine reaction order made it obvious that the reaction studied did not follow one of the simpler kinetic relationships. The curves obtained, illustrated in Fig. 1, resembled typical autocatalytic plots, but an attempt to establish autocatalysis by graphical means gave negative results. Therefore, a mechanism was postulated based on the known physical and chemical conditions of the reaction.

The proposed mechanism is based on the following tenets: (a) Water is rapidly adsorbed onto the surface of the aspirin, the amount of water being a function of vapor pressure. If multilayer adsorp-

TABLE I.—DECOMPOSITION OF ASPIRIN AT VARIOUS TEMPERATURES AND VAPOR PRESSURES

50°					
46.02 mm.		54.40 mm.		68.30 mm.	
Time in Days	Mole % Aspirin	Time in Days	Mole % Aspirin	Time in days	Mole % Aspirin
0.0	99.90	0.0	99.90	0.0	99.90
3.0	99.87	3.0	99.86	3.0	99.84
24.0	99.83	24.0	99.70	7.0	99.60
34.1	99.66	34.0	99.43	21.9	99.22
40.9	99.53	40.9	99.27	30.0	99.56
47.9	99.29	47.9	98.83	34.1	99.05
61.9	99.26	61.7	98.98	40.9	98.97
76.8	98.73	76.8	98.26	47.9	98.69
103.8	98.51	103.8	97.83	61.9	98.10
139.8	97.77	139.8	96.91	76.8	97.40
170.8	96.86	170.8	94.69	103.8	95.98
...	139.8	93.82
...	170.8	92.64
...
60°					
68.41 mm.		74.50 mm.		111.9 mm.	
Time in Days	Mole % Aspirin	Time in Days	Mole % Aspirin	Time in Days	Mole % Aspirin
0.0	99.90	0.0	99.90	0.0	99.90
10.8	99.42	2.8	99.70	2.9	99.60
20.0	99.00	10.8	99.34	6.8	99.07
27.7	98.27	15.8	98.71	10.8	99.13
33.8	97.91	23.8	98.54	15.8	98.20
44.7	96.84	27.7	97.73	20.7	97.50
48.7	96.35	37.7	97.00	23.8	97.21
51.8	95.94	41.7	96.64	27.7	96.21
61.8	94.80	44.7	95.66	30.8	96.02
83.6	91.38	51.8	95.08	33.8	94.91
99.1	88.50	61.8	93.20	37.8	95.15
120.0	85.26	72.8	91.81	41.7	94.06
141.0	80.74	83.6	89.97	44.7	93.86
...	...	99.1	85.66	48.7	91.11
...	...	107.7	83.65	51.8	90.66
...	...	120.0	79.99	55.7	89.32
...	...	141.0	75.95	61.8	81.46
...	72.8	79.51
...	83.6	73.09
...	91.7	68.66
...	107.7	65.50
...	114.7	60.16
...	120.0	60.03
...	132.0	54.98
...
...
80°					
181.0 mm.		199.5 mm.		232.5 mm.	
Time in Hr.	Mole % Aspirin	Time in Hr.	Mole % Aspirin	Time in Hr.	Mole % Aspirin
0.0	99.90	0.0	99.90	0.0	99.90
17.0	99.68	17.5	99.39	17.3	99.25
40.8	99.69	40.3	98.16	40.5	98.64
63.3	97.96	62.5	97.41	63.3	97.05
90.5	95.79	90.0	95.24	90.3	94.40
112.5	94.45	111.8	92.39	112.8	86.75
137.0	91.77	136.3	87.80	136.8	84.78
160.5	88.79	160.0	85.69	160.8	77.44
186.5	85.28	186.0	73.86	186.2	72.80
208.8	83.34	208.3	78.40	209.8	67.54
232.8	81.96	232.5	57.31	232.8	60.76
308.8	70.86	308.3	50.20

tion takes place a film of water will surround each aspirin particle, and the thickness of film will depend on vapor pressure. If monolayer adsorption takes place, each aspirin particle will be partially covered by a layer of water, the extent of coverage being dependent on vapor pressure. (b) The water

film is rapidly saturated by solution of a portion of the solid aspirin. Decomposition then occurs in solution, and as a molecule of aspirin is removed via hydrolysis it is instantly replaced by one from the solid. Although only a small portion of the aspirin is

actually in solution, the reaction may be treated as though all the aspirin is in solution and only a small fraction is in active state. This makes it possible to express the concentration in terms of total amount of solid aspirin.

Treatment of this system as a solution makes it possible to use one of the rate constants derived by Edwards (12). The constants of Edwards depending on water concentration are so small as to be negligible, while the concentration of hydroxyl ion in this system is so low that the extent of reaction dependent on it is negligible. Further, since the ionization constant for aspirin is small and ionization would be depressed by hydrogen ion concentration, it is believed that this system involves primarily molecular aspirin. Thus, only one of the constants described by Edwards, k_1 , which is associated with hydrogen ion concentration and molecular aspirin, is necessary for the rate expression for this system.

Accordingly the rate equation may be written: $dC/dt = k_1(A)(H^+)$, (Eq. 1), in which C is the concentration of salicylic acid, A is the concentration of aspirin, and H^+ is the concentration of hydrogen ion. Using the definition of the ionization constant for acetic acid, $K = [(H^+)(CH_3COO^-)]/(CH_3COOH)$, (Eq. 2), the concentration of salicylic acid, C , may be substituted for that of acetic acid, since one mole of acetic acid is formed for each mole of salicylic acid, and the decrease in concentration of acetic acid due to ionization is negligible. Also, the concentration of hydrogen ion is equal to that of acetate ion. Thus, the ionization equation may be stated: $K = (H^+)^2/(C)$ (Eq. 3).

In order to convert concentrations to the number of moles contained in volume of solution, V , on the surface of the particles, K , which has units of moles/liter, is multiplied by V to give: $KV = (H^+)^2/(C)$, (Eq. 4). Solving for hydrogen ion concentration, $[H^+] = (KV[C])^{1/2}$, (Eq. 5); and substituting Equation 5 in Equation 1 gives $dC/dt = k_1[A](KV[C])^{1/2}$, (Eq. 6), which applies to any single vapor pressure, and has units of moles $\times V^{-1} \times t^{-1}$. To express the rate in terms of number of moles rather than concentration the equation is multiplied by V , to give $dC/dt = k_1K^{1/2}V^{1/2}[A][C]^{1/2}$, (Eq. 7).

It should be noted that although the units of dC/dt are moles $\times t^{-1}$ those of $[A]$ and $[C]$ are moles $\times V^{-1}$. Since aspirin samples have different weights they are converted to a common basis by expressing both $[A]$ and $[C]$ as per cent of the total number of moles originally present, giving them the units mole% $\times V^{-1}$.

It is possible to express aspirin concentration in terms of salicylic acid concentration, since their sum is always a constant equal to 100. Thus, $[A] + [C] = [A_0] + [C_0] = D_0$, (Eq. 8), and $[A] = D_0 - [C]$, (Eq. 9), where A_0 and C_0 are initial concentrations of aspirin and salicylic acid. This substitution facilitates integration to give $dC/dt = k_1K^{1/2}V^{1/2}(D_0 - [C])[C]^{1/2}$, (Eq. 10).

The volume of the solution layer, V , is unknown, but it may be expressed in terms of the Freundlich isotherm equation; thus, for a particular size and shape of particle: $V = k'p^n$, (Eq. 11), in which p is the vapor pressure, k' is the proportionality constant, and n is the order of the sorption reaction with respect to p . Substituting this expression for V into Equation 10: $dC/dt = kp^{3n/2}(D_0 - [C])$

$[C]^{1/2}$, (Eq. 12), where $k = k_1K^{1/2}k'^{n/2}$. By making the substitution $[C]^{1/2} = y$ the following equation is obtained: $2dy/(D_0 - y^2) = kp^{3n/2} dt$, (Eq. 13), which can be readily integrated by standard form, giving:

$$\frac{1}{D_0^{1/2}} \ln \frac{(D_0^{1/2} + [C]^{1/2})}{(D_0^{1/2} - [C]^{1/2})} = kp^{3n/2}t + I \quad (\text{Eq. 14})$$

From Equation 9 it follows that $(D_0^{1/2} + [C]^{1/2})(D_0^{1/2} - [C]^{1/2}) = [A]$; inserting this into Equation 14:

$$\frac{1}{D_0^{1/2}} \ln \frac{(D_0^{1/2} + [C]^{1/2})^2}{[A]} = kp^{3n/2}t + I \quad (\text{Eq. 15})$$

Setting t equal to zero, the constant of integration, I , is found to be:

$$\frac{1}{D_0^{1/2}} \ln \frac{(D_0^{1/2} + C_0^{1/2})^2}{A_0} \quad (\text{Eq. 16})$$

Rearranging and converting to logarithms to the base 10, the final equation is obtained:

$$\log \frac{A_0(D_0^{1/2} + C^{1/2})^2}{(D_0^{1/2} + C_0^{1/2})^2 A} = \frac{D_0^{1/2}kp^{3n/2}t}{2.303} \quad (\text{Eq. 17})$$

If the mechanism proposed is correct, a plot of the left hand side of Equation 17 versus time should give a straight line with a slope equal to $(D_0^{1/2}kp^{3n/2})/2.303$. The data of Table I were plotted in this manner and found to give straight lines. Figure 2 illustrates this type of graph. The values of the slopes of the lines obtained in this manner are shown in Table II.

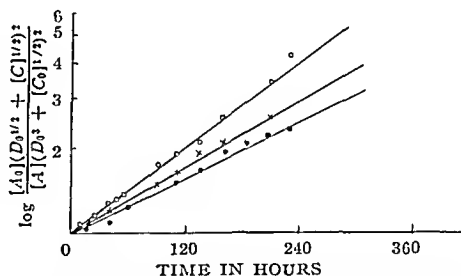


Fig. 2.—Typical plots of decomposition equation (Eq. 17), 80°. Legend: O—vapor pressure 232.5 mm.; x—vapor pressure 199.5 mm.; ●—vapor pressure 181.0 mm.

It can be seen from the equation of the slope that a plot of $\log (D_0^{1/2}kp^{3n/2})/2.303$ versus $\log p$ should give a straight line with a slope of $3n/2$ and an intercept of $\log D_0^{1/2}k/2.303$. These graphs are shown in Fig. 3, and the values of the slopes and intercepts are presented in Table III.

From chemisorption theory (15) n would be expected to be 1, making $3n/2$ equal to 1.5. The results of Table III are in agreement with this within experimental error; thus, the intercepts of Table III were calculated using a value of 1.5 for $3n/2$.

In order to estimate the activation energy for the reaction, k , which is the product of k_1 , $k'^{1/2}$ and $K^{1/2}$,

TABLE II.—SLOPES OF THE DECOMPOSITION EQUATION OF ASPIRIN (Eq. 17)

Temp. °C.	Vapor Pressure mm.	Slope = $D_0^{1/2} k p^{3n/2} / 2.303$
80	181.0	$1.660 \times 10^{-3} \text{ hours}^{-1}$
	199.5	$1.986 \times 10^{-3} \text{ hours}^{-1}$
	232.5	$2.475 \times 10^{-3} \text{ hours}^{-1}$
60	68.41	$2.772 \times 10^{-3} \text{ days}^{-1}$
	74.50	$3.191 \times 10^{-3} \text{ days}^{-1}$
	111.9	$5.218 \times 10^{-3} \text{ days}^{-1}$
	120.3	$7.547 \times 10^{-3} \text{ days}^{-1}$
50	46.02	$7.558 \times 10^{-4} \text{ days}^{-1}$
	54.50	$9.980 \times 10^{-4} \text{ days}^{-1}$
	68.30	$1.367 \times 10^{-3} \text{ days}^{-1}$
	74.20	$1.860 \times 10^{-3} \text{ days}^{-1}$

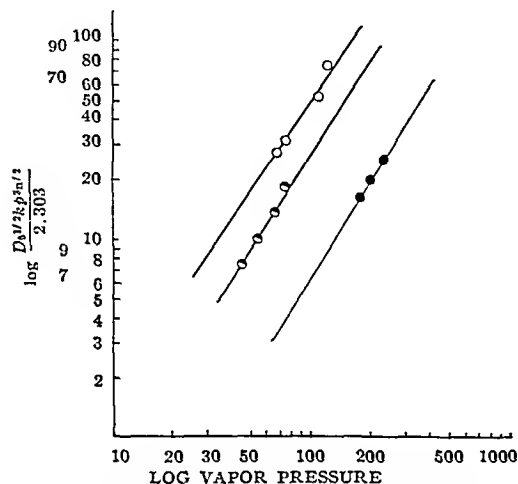


Fig. 3—Plots to determine order of vapor pressure effect. Legend: ○—60°; ◐—50°; ●—80°.

TABLE III

Temp. °C.	$3n/2$	$D_0^{1/2} k / 2.303$
80	1.5	$6.953 \times 10^{-7} \text{ mm.}^{-1/2} \text{ hrs.}^{-1}$
60	1.5	$4.976 \times 10^{-6} \text{ mm.}^{-1/2} \text{ days}^{-1}$
50	1.7	$2.552 \times 10^{-6} \text{ mm.}^{-1/2} \text{ days}^{-1}$

TABLE IV. DECOMPOSITION RATE CONSTANTS FOR ASPIRIN AT VARIOUS TEMPERATURES

Temp. °K	$k_1 k^{1/2}$ (Mole %) ^{-1/2} Liters ^{1/2} Moles ^{-1/2} mm. ^{-1/2} Hr. ⁻¹
353.1	44.58×10^{-6}
333.1	12.41×10^{-6}
323.1	6.06×10^{-6}

is divided by $K^{1/2}$, the value of which is known for each of the temperatures studied (16). This leaves $k_1 k^{1/2}$ the values of which are shown in Table IV. From a plot of $\log k_1 k^{1/2}$ versus $1/T$ the activation energy can be obtained. This value is the sum of two such energies, one associated with the chemical hydrolysis reaction, and the other with the physical sorption reaction. However, since there is no rea-

son to believe that the sorption forces are other than van der Waals, the energy of activation for this reaction can be considered zero (15). Thus the activation energy determined from the slope of an Arrhenius plot is that of the hydrolysis reaction, and was calculated to be 15,065 calories per mole, which is in good agreement with the value of 15,620 determined by Edwards (17).

DISCUSSION

It should be noted that the method used in this work for control of humidity is quite troublesome. The normal variations in temperature of laboratory ovens is sufficient to cause condensation of water on the inside of the desiccators which frequently causes loss of a whole series of samples. Also, data obtained once the per cent aspirin remaining has dropped to a low value, usually around 20–30%, is widely scattered. At this point the samples are commonly observed to become sticky and aggregate or even form a semisolid mass. It is believed that a different physical system exists at this point. Data obtained under such conditions are not reported in this paper and do not follow the mechanism proposed. However, decomposition at this stage is of minor importance.

By use of the equations established in this paper, it is possible to predict the stability of aspirin under known conditions of temperature and humidity. Application of the equation is limited, however, to the particle size used in this work. Further work is in progress in this laboratory to determine the relationship between particle size and rate constant.

It appears that by knowing the ionization constant of a material to be mixed with aspirin, one might use these equations to predict the stability of the mixture. Other factors such as solubility of the added substance will have to be taken into account. Work of this type is planned.

The basic assumptions and treatment of data used in this study show promise of being applicable to many solid medicinals which decompose as a result of adsorption of water.

REFERENCES

- (1) Tsakalotos, D. E., *J. Pharm. Chim.*, **14**, 174 (1916).
- (2) Paolini, V., *Giorn. chim. ind. ed. appl.*, **3**, 403 (1921); *Chem. Abstr.*, **16**, 3147 (1922).
- (3) Stathopoulos, T. G., *Prak. Alkad. Athēnōn*, **6**, 229 (1931); *Chem. Abstr.*, **27**, 2250 (1933).
- (4) Ribeiro, D., Stevenson, D., Samyn, J., Milosovich, G., and Mattocks, A. M., *This Journal*, **4**, 226 (1955).
- (5) Ebert, W. R., Ph. D. Thesis, University of Michigan (1956).
- (6) Yamamoto, R., and Takahashi, T., *Ann. Repts. Shionoga Research Lab.*, **3**, 79 (1954).
- (7) *Ibid.*, **3**, 112 (1953).
- (8) Tsakalotos, D. E., and Horsch, S., *Bull. Soc. Chem.*, **15**, 743 (1914); **17**, 186 (1915); **17**, 401 (1915).
- (9) Morton, C., *Quart. J. Pharm. and Pharmacol.*, **6**, 492 (1934).
- (10) Ferroni, E., and Baistrocchi, R., *Sperimentale. Sez. chem. biol.*, **4**, 7 (1953); *Chem. Abstr.*, **47**, 10974h (1953).
- (11) Sturtevant, J. M., *J. Am. Chem. Soc.*, **64**, 723 (1950).
- (12) Edwards, L. J., *Trans. Faraday Soc.*, **46**, 723 (1950).
- (13) "Methods of Analysis of the A. O. A. C.," 6th ed., A. O. A. C., Washington, D. C., 1945, p. 673.
- (14) Stokes, R. H., and Robinson, R. A., *Ind. Eng. Chem.*, **41**, 2013 (1953).
- (15) Laidler, K. J., "Chemical Kinetics," 1st ed., McGraw-Hill, New York, 1950, p. 146.
- (16) "International Critical Tables," 1st ed., McGraw-Hill, New York, 1929, p. 262.
- (17) Edwards, L. J., *Trans. Faraday Soc.*, **48**, 696 (1952).

The Dispersion of Liquids in Aqueous Solutions of Amphiphilic Compounds*

By WILLIAM J. O'MALLEY,[†] LUCIANO PENNATI,[‡] and
ALFRED N. MARTIN

The influence of certain amphiphilic compounds (molecules possessing both hydrophilic and lipophilic character) on the dispersion of nonpolar substances in water is presented in the form of ternary phase diagrams. The dispersion systems studied were peppermint oil-water-Tween 20, peppermint oil-water-polyethylene glycol 400, and benzyl benzoate-water-polyethylene glycol 400. The data were plotted as triangular diagrams to show the effects of the three agents simultaneously. The results suggested that such a method should assist one in the formulation of concentrates for the preparation of aromatic waters and other solubilized products.

NORMALLY INSOLUBLE liquids and solids may be dispersed in aqueous systems by several means, principally among which are solubilization, co-solvency or blending, and emulsification.

These processes are made possible by the presence of an amphiphilic compound, i. e., a substance which has a tendency to a greater or a lesser degree to dissolve in water (hydrophilic character) and in nonpolar solvents (lipophilic or organophilic character). Amphiphilic compounds include solubilizing agents, detergents, emulsifiers, and solvents of a polar-nonpolar nature (1).

McBain (2) defined solubilization as the dispersion of relatively insoluble matter in aqueous solutions of amphiphiles to form thermodynamically stable systems. Above a minimum amount, known as the critical micelle concentration, the amphiphilic compound exists in solution in the form of micelles. Normally insoluble liquids or solids are adsorbed on the surface of the micelles, pass into the micelle interior and dissolve in the nonpolar region, or are adsorbed in the palisade layer of the micelle (3).

Blending and co-solvency are terms used to describe the mutual solubility of normally immiscible liquids in the presence of an amphiphilic agent. Sodium oleate, for example, increases the co-solvency of benzene and propylene glycol, presumably through the formation of bridges between the polar and nonpolar molecules (4).

Emulsification is familiar to every pharmacist; in this discussion it is considered in relation to solubilization. When oil is added to a solution of a solubilizing agent it continues to enter the micelle, which consequently swells un-

til saturation is reached. Beyond this point, which is marked by the appearance of turbidity (the cloud point), the system becomes an emulsion of finely dispersed oil globules surrounded by amphiphilic molecules. Accordingly, emulsification may be looked upon as an extreme case of solubilization, which begins when sufficient oil has been added to form microscopic droplets that exceed the limit of solubilization (3).

The polyoxyethylene derivatives of sorbitan (the Tweens) are solubilizing agents, while the polyethylene glycols may be referred to as blending agents. However, since both may be used to bring about the co-solvency of water and nonpolar liquids, Tween 20 and polyethylene glycol 400 will both be called blending agents in the following discussion.

A number of reports have been published during the last decade on the use of various amphiphilic agents for the dispersion of certain pharmaceutical substances, and they have stressed the advantages of these agents in forming clear, stable solutions of normally insoluble drugs. Monte Bovi (5) used Tween 20 to make a peppermint oil concentrate; Ohmart and Stoklosa (6) discussed some pharmaceutical applications of the Tween and Spans; Nakagawa (7) used the esters of polyoxyethylene to solubilize hormones and vitamins; Loran and Guth (8) studied the use of ethyl alcohol for blending water and castor oil.

The present study was directed toward investigating the dispersion of several nonpolar liquids of pharmaceutical interest in water by means of two amphiphilic agents, namely, Tween 20¹ and polyethylene glycol 400.² The effect of dilution and temperature variation was also considered in the Tween 20 system.

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¹ Supplied by the Atlas Powder Company, Wilmington, Del.

² Supplied by the Carhide and Carbon Chemicals Company, New York, N. Y.

EXPERIMENTAL

The ternary systems studied were: (a) peppermint oil-water-Tween 20, (b) peppermint oil-water-polyethylene glycol 400, and (c) benzyl benzoate-water-polyethylene glycol 400

The specific gravities of the ingredients were determined by using a 25-ml. pycnometer at 20°, and the values were found to be as follows: peppermint oil, 0.898, benzyl benzoate, 1.115; Tween 20, 1.102; polyethylene glycol 400, 1.120.

The samples were prepared by pipetting a definite volume of the substance to be solubilized into a 125-ml flask and adding a definite volume of the solubilizing agent. The solutions were mixed by means of a magnetic stirrer at a slow speed to prevent the formation of air bubbles. The samples were titrated with distilled water until the entire solution remained turbid (cloud point) for one minute as observed in a beam of light passing through the solution perpendicular to the line of vision. The solutions were not allowed to come to equilibrium since this may take several weeks (9, 10). The turbidity, which formed immediately, was taken arbitrarily as the end point, recognizing that it did not

to weight using the specific gravities, and the weights of the three ingredients in a given sample were taken to equal 100 per cent

RESULTS

The results for the system of peppermint oil-water-Tween 20 were plotted as seen in Figs. 1, 2, 3 and 4; and the other systems are shown in Figs. 5 and 6. The volumes of the three ingredients were converted to weight by using specific gravities. Table I gives the weights of the three ingredients necessary to cause a change of phase as is seen in Fig. 1. Regions I/II, II/III and III/IV in Table I represent the limiting values for a visible change of phase from one region to the next. The two curves shown in Fig. 1 represent the per cent by weight and the per cent by volume of the three ingredients.

The Effects of Dilution on the Peppermint Oil-Water-Tween 20 System.—Figure 2 has been used to show some of the difficulties that might accompany the use of surface-active agents to make solubilized concentrates. In this reproduction of a portion of Fig. 1 from zero to 30 per cent peppermint oil, *A* represents a peppermint oil concentrate con-

TABLE I.—THE WEIGHT PER CENT DATA FOR THE SYSTEM OF PEPPERMINT OIL (O), WATER (W), TWEEN 20 (T) AT 20° NECESSARY TO BRING ABOUT A CHANGE FROM ONE PHASE TO THE NEXT

Region I/II			Region II/III			Region III/IV		
O	T	W	O	T	W	O	T	W
						1.5	8.7	89.8
						3.2	16.3	80.5
						9.0	30.3	60.7
						11.5	35.3	53.2
24.5	67.4	8.1	23.7	65.2	11.1	13.6	37.4	49.0
29.3	66.6	6.1	25.4	62.0	12.6	16.4	40.2	43.4
30.4	64.8	4.8	27.8	59.2	13.0	19.5	41.7	38.8
33.9	62.1	4.0	30.8	56.5	12.7	23.2	42.5	34.3
38.2	58.4	3.4	34.5	52.7	12.8	28.2	43.2	28.6
43.8	53.6	2.6	39.9	48.7	11.4	34.8	42.4	22.8
46.4	51.3	2.3	42.3	46.7	11.0	38.0	42.1	19.9
50.3	46.1	3.6	46.3	42.4	11.3	43.8	40.3	15.9
55.9	41.0	3.1	52.6	38.6	8.8	50.4	37.0	12.6
60.0	36.7	3.3						..
64.7	31.6	3.7						
71.8	26.3	2.9						
82.5	15.1	2.4						

represent the equilibrium value but rather supplied a point which could be reproduced upon subsequent tests.

The cloud point was considered as the transition between a solubilized and an emulsified system when the solubilizing agent was used. Although clouding does not necessarily indicate a distinct separation of two phases, it suggests a state of instability, and is referred to later in connection with the phase diagrams as the beginning of a *region of immiscibility*.

The peppermint oil-water-Tween 20 system was studied at temperatures of 10°, 20°, 30°, and 40° using a constant temperature bath to maintain the temperature within $\pm 0.1^\circ$. The other systems were investigated at 20°. The volumes were converted

to weight using the specific gravities, and the weights of the three ingredients in a given sample were taken to equal 100 per cent. The plot indicates that such a concentrate is satisfactory for the preparation of an aromatic water at 25°.

In Fig. 2, concentrate *B*, was used to point up the difficulties that might result from an indiscriminate use of surface-active agents. For example, when 49% peppermint oil, 50% Tween 20 and 1% water were combined a clear solution *B* was produced, but when 10.0 ml. of this solution were diluted with 0.1 ml. of distilled water, a cloudy mixture *B'* resulted. When 1.0 ml. more of distilled water was added,

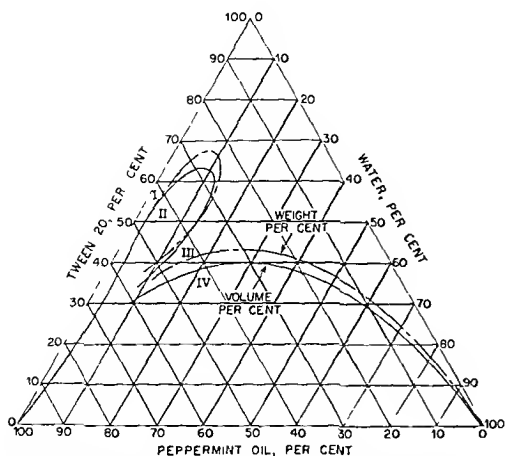


Fig. 1.—The three-component phase diagram showing regions of miscibility and immiscibility at 20°, and comparing weight per cent and volume per cent for peppermint oil, Tween 20 and water.

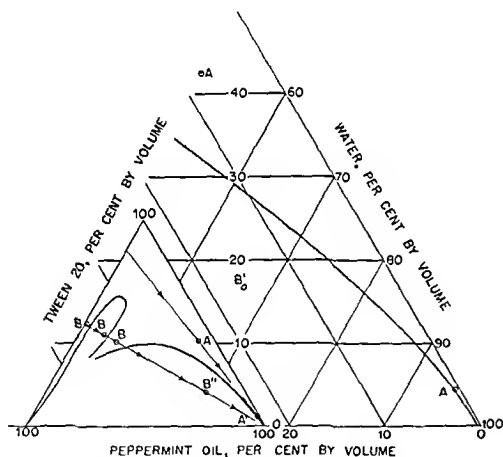


Fig. 2.—Enlargement of a portion of Fig. 1 showing regions of miscibility and immiscibility at 20°. The effects of diluting volatile concentrates for the preparation of aromatic waters are observed by following the lines through points A and B on the insert diagram.

the mixture again became clear as represented by point B'', but when the total volume was brought to 30.0 ml., a turbid mixture B''' was formed, and it remained cloudy until a point was reached where the concentration was approximately equal to the solubility of peppermint oil in water.

In Fig. 3, the grams of oil solubilized were plotted *versus* grams of surface-active agent used. The region plotted covers the same concentrations as Fig. 2 but shows more clearly the solubility of the oil as a function of the amphiphilic agent.

The Influence of Temperature.—Figure 4 depicts the effects of changes in temperature upon the solubility of the peppermint oil-water-Tween 20 mixture.

With a decrease in temperature the region of immiscibility below the curve (Region IV) increased while Region II decreased in size. At 10° the mixture was very viscous, and it was not possible to obtain an unambiguous cloud point.

Other Ternary Systems.—Figure 5 shows the phase diagram relationship for peppermint oil-

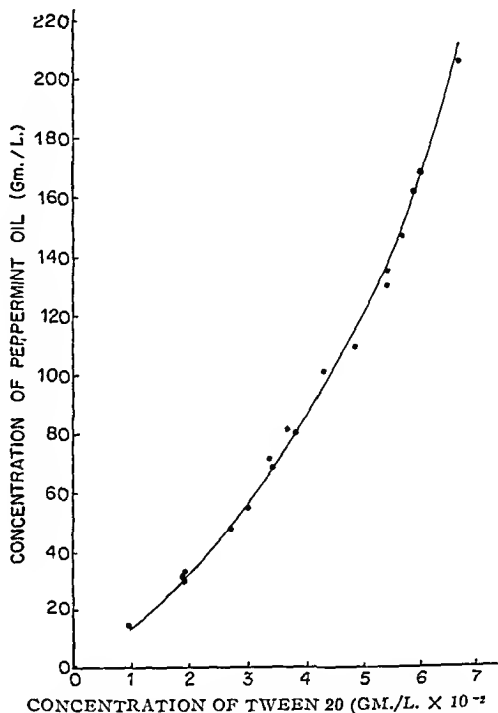


Fig. 3.—The solubilization of peppermint oil with Tween 20 in water.

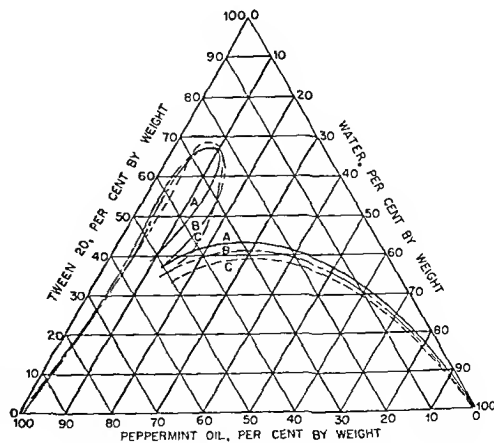


Fig. 4.—Three-component phase diagram showing regions of miscibility and immiscibility at various temperatures for peppermint oil, Tween 20 and water. A, 20°, B, 30°, C, 40°.

water-polyethylene glycol 400, and Fig. 6 illustrates the blending of benzyl benzoate and water by the use of polyethylene glycol 400. The area above the curve in each case represents a clear solution, whereas, the area under the curve is a two-phase region of

immiscibility. The differences between the character of the results in Fig 2 and Figs 5 and 6 point up the fact that the oil is probably brought into solution by different mechanisms. The solubility shown in Figs 5 and 6 involves the action of a true co-solvent rather than a micelle-forming surface-active agent

SUMMARY AND CONCLUSIONS

Ternary phase diagrams have been used in this study to describe the pharmaceutical systems containing peppermint oil, water and an amphiphilic agent, and they can assist one in the development of solubilized products.

The arrows drawn through the points *A* and *B* in Fig 2 indicate how the concentrations of the ingredients change upon dilution with water.

Care must be exercised in formulating a solubilized oil concentrate so that a turbid preparation will not result on dilution. The influence of temperature on the mixture was also studied. Moderate changes in temperature have little effect on the phase equilibrium; however, a decrease in ambient temperature may produce turbidity in a normally clear solubilized system.

By comparing Figs 1 and 5, it can be seen that Tween 20 is more efficient in blending peppermint oil and water than is polyethylene glycol 400. The diagrams also suggest that peppermint oil is probably brought into solution by these two amphiphilic agents by different mechanisms.

REFERENCES

- (1) Winsor, P A, *Trans Faraday Soc*, **44**, 376(1948). "Solvent Properties of Amphiphilic Compounds" Butterworths Scientific Publications, London, pp 55-61, *Mfg Chemist*, **27**, 89(1956)
- (2) McBain, M E, and Hutchinson, Eric "Solubilization" Academic Press Inc, Publishers, N Y, 1955, p xi
- (3) Kleven, H B, *Chem Revs*, **47**, 1(1950)
- (4) McBain, J W, and Palit, S R, *Ind Eng Chem*, **38**, 741(1946)
- (5) Monte Bovil, A J, *THIS JOURNAL, Pract Pharm Ed*, **11**, 107(1950), *ibid*, **12**, 565(1951)
- (6) Omhart, L M, and Stoklosa, M J, *ibid*, **12**, 23(1951)
- (7) Nakagawa, Toshio, *J Pharm Soc Japan*, **73**, 469, 732, 1016(1953), *ibid*, **74**, 858(1954), through *Chem Abstr*, **47**, 9563, 11661(1953), *ibid*, **48**, 1631 12374(1954)
- (8) Loran, M R, and Guth, E P, *THIS JOURNAL*, **40**, 465(1951)
- (9) Lambert, J M, and Bussee, W F, *J Chem Phys*, **16**, 847(1948)
- (10) McBain, W. J., et al, *J Am Chem Soc*, **66**, 9(1944)

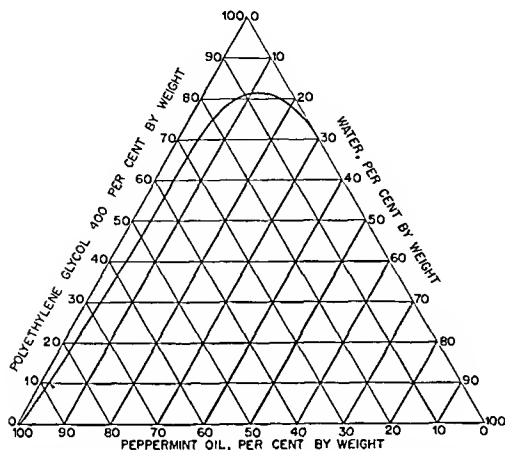


Fig 5—Three-component phase diagram showing the regions of miscibility and immiscibility at 20° for peppermint oil, polyethylene glycol 400 and water

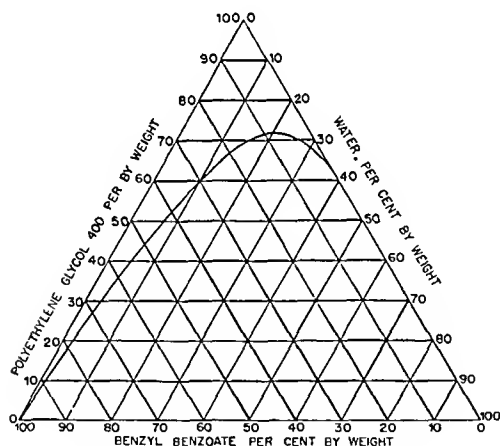


Fig 6—Three-component phase diagram showing the regions of miscibility and immiscibility at 20° for benzyl benzoate, polyethylene glycol 400 and water.

The Effect of Nicotine on Experimental Hypercholesterolemia in the Rabbit*

By DUANE G. WENZEL† and GERALD L. BECKLOFF

The administration of 2.28 mg./Kg./day of nicotine alkaloid in the drinking water of rabbits fed a 0.1% cholesterol diet significantly increased the plasma total cholesterol levels over those of control groups. Cholesterol/lipid phosphorus (C/P) ratios, which may be used as an indication of atherogenic susceptibility, were not raised to the same extent because of concomitant increases in lipid phosphorus. Significant differences in serum ascorbic acid, serum stability, bromsulfalein retention, aortic cholesterol content, and aortic plaques could not be demonstrated at terminal determinations possibly because of reduced group sizes.

THE MEDICAL LITERATURE is replete with investigations concerning the effects of nicotine on the cardiovascular system. Clinically it is generally conceded that nicotine should be avoided in vascular diseases such as thromboangiitis obliterans (1), Raynaud's syndrome (2), peripheral arteriosclerosis (3), and its use is inadvisable in the cardiac sensitivity known as tobacco angina (4). These untoward effects of nicotine are probably related to the decreased peripheral blood flow produced by nicotine as demonstrated by skin temperature fall (5), plethysmographic studies (6), and visual observation of capillary blood flow (7).

While it is apparent that the vasoconstrictive effect of nicotine is detrimental to most of the peripheral vascular diseases, it is difficult to relate its action directly to atherosclerosis. It appears to be more or less tacitly assumed that if nicotine is related to atherosclerosis this relationship is not causal in nature but that its action, at most, only aggravates the already existing disease.

Thienes and Butt (8) treating both rats and rabbits with nicotine found an apparent lack of cardiovascular toxicity as a greater percentage of degenerative vascular changes occurred in the controls than in the experimental group. In a clinical study of 301 male diabetics, however, it was reported 53% of the smokers suffered from atherosclerosis as compared to 23% in the nonsmokers (9). Swiss investigators directed attention to the fact that only 6.7% of a large group of coronary patients were nonsmokers as compared to 25.5% of nonsmokers in a comparable control group (10). They further established that there were more heavy smokers among the athero-

sclerotic patients (45%) than the control (28.5%) and the heavier the smoking the younger the age of onset. In a study of 1,520 patients with angina pectoris and coronary thrombosis, Sigler (11) concluded that there was a direct correlation between the amount of smoking and both the early appearance of the first coronary occlusion and the occurrence of death due to this cause. Hammond and Horn (12) in a follow-up study of 190,000 men found that the death rate from coronary artery disease was almost twice as high for men smoking one or more packs of cigarettes daily than for nonsmokers.

As coronary artery disease is usually atherosclerotic in nature it is difficult to account for the increased death rate on the basis of a vasoconstrictive action alone. In fact, smoking has been stated to be of no direct danger to the cardiac patient through coronary vasoconstriction (13). Barger, *et al.* (14), observed that while smoking one cigarette produces an average increase of 19% in coronary blood flow, myocardial oxygen consumption rises by about 27%. In rabbits, nicotine reduces the coronary blood flow in atherosclerotic Langendorff hearts (15).

One possibility is that some of the circumstantial evidence for the role of dietary fats and cholesterol in the production of atherosclerosis may be related in part to nicotine. For example, it has been observed that a steady increase in coronary artery disease occurred in Norway until 1940, the time of the German occupation. During the occupation when the average daily fat intake was reduced from the usual 159 grams to 71 grams, there was essentially a corresponding decrease in deaths due to circulatory diseases. While the probable role of fats in the changing picture is not denied, it must also be considered that during this same period the tobacco consumption was at a relatively low level (16). A similar possibility exists in studies which have related the low fat intake of certain primitive so-

* Received July 10, 1957 from the School of Pharmacy, University of Kansas, Lawrence.

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cetics to reduction in cardiovascular disease (17). It is likely that here again the low fat diet is accompanied by minimal tobacco consumption.

The following work was designed as a pilot study to explore the possibility of a direct relationship between nicotine and the plasma cholesterol level. Although the atherosclerotic human does not necessarily have hypercholesterolemia, individuals having evidence of coronary disease as a group tend to have higher than normal serum cholesterol levels (18, 19). Experimentally induced atherosclerosis of animals is usually preceded and (or) accompanied by hypercholesterolemia but can be induced by small doses of cholesterol producing minimal hypercholesterolemia (20).

This initial investigation is limited to the examination of the role of nicotine in total plasma cholesterol and lipid phosphorus levels and to the factors which may be responsible for changes observed.

EXPERIMENTAL

Methods.—Male New Zealand white rabbits were used in this study. The animals were six weeks of age at the beginning of the problem and weighed between 2012 and 2333 grams. Group I, the untreated control, was fed Purina Rabbit Chow Checkers and water *ad libitum*. Group II, the cholesterol control, received the same diet except for the addition of 0.1% cholesterol to the feed. This was added by dissolving the cholesterol in sufficient chloroform to uniformly moisten the pellets during thorough mixing. The chloroform was then removed by evaporation in a steam oven. It should be noted that 0.1% cholesterol in the diet is a smaller quantity than that usually administered. A 1% cholesterol diet is ordinarily considered to produce maximal hypercholesterolemia, but the 0.1% level was selected in order to be able to more readily observe possible increases in the plasma cholesterol level. Group III, the nicotine control, received the control diet plus a measured amount of nicotine alkaloid in the drinking water. The amount of nicotine was calculated to supply a quantity of the alkaloid equivalent on a weight basis to the human consumption of two packs of cigarettes daily. The average daily water consumption per rabbit was 350 ml. Four milligrams of nicotine *per os* has been reported to produce the same psychic effects as smoking one cigarette by a habitual smoker (21). Using 70 Kg. as the average adult human weight, the daily two pack rabbit equivalent is approximately 2.28 mg./Kg. of nicotine alkaloid. This quantity was the final daily amount of nicotine in each 350 ml. drinking water of groups II and IV. All drinking containers were plastic. Group IV had the combined treatments of groups II and III; that is, both cholesterol and nicotine.

In order to reduce the possibility of acute nicotine toxicity, the production of tolerance was attempted by gradually increasing the size of the dose. For the first three days the nicotine equivalent of 3.33

"rabbit cigarettes" was given daily. This same quantity was added to the total daily intake at the end of each three-day period until at the end of thirty-six days the full 40 "rabbit cigarette" equivalent was being administered. The quantity of nicotine was adjusted throughout the experiment to correspond to the increase in body weight.

Immediately prior to the initiation of the study, total plasma cholesterol and lipid phosphorus levels were determined for all animals. These were repeated at intervals of four weeks for the twenty-week test period. Total plasma cholesterol was determined by a modification of the Bloor-Sackett method (22). Plasma lipid phosphorus levels were established by a modified Youngberg procedure (23).

At the termination of the test period additional tests were conducted. A serum ascorbic acid analysis was made of each animal according to Lowry (24). The relative serum stability was determined according to a modification of the work of Ressler, *et al.* (25). Using their technique on hypercholesterolemic serum resulted in spectrophotometric readings too turbid to read. Since the results are simply comparative for the control and experimental groups, the following procedure was used which appeared to allow adequate light transmission. Two-tenths milliliter of 0.006 *M* zinc acetate solution was added to 1.5 ml. of serum at thirty-second intervals until a total of 1.8 ml. had been added. Two-tenths milliliter of water was then added and readings were made at 510 $m\mu$ with a zero setting at 70% transmission. Readings were as low as 21% transmission, yet a number reached over 110% and could not be considered as true numbers. A bromsulfalein test for liver function was conducted according to the usual procedure (26).

When the preceding tests were completed the animals were sacrificed and the aortas graded for gross atherosclerotic lesions by the method of Horlick and Katz (27). After grading, the aortas from the left carotid to the right renal artery were dried over potassium hydroxide, extracted as directed by Faber (28) and the cholesterol determined by the same method as used in the plasma cholesterol determinations. Liver and body weights were also recorded.

RESULTS AND DISCUSSION

Table I and Fig. 1 show that the plasma cholesterol levels were approximately equal for all groups at the beginning of the experiment. While the cholesterol levels of the nicotine and control groups then fell slightly and remained relatively constant throughout the twenty weeks of the experiment, both the cholesterol and nicotine-cholesterol groups had an immediate increase in plasma cholesterol at four weeks with leveling at the eight-week test. The nicotine-cholesterol group, however, had a further increase between the eight- and twelve-week periods with subsequent leveling.

The amount of plasma lipid phosphorus was also approximately the same for all four groups at the onset as seen in Table I and Fig. 2, but the changes differed from those of the plasma cholesterol. The nicotine and control group levels showed a falling trend while both the cholesterol and nicotine-cholesterol rabbits had a rise at four weeks with a fall in the levels of the cholesterol group after this test. The lipid phosphorus values of the nicotine-chole-

TABLE I.—MEAN TOTAL CHOLESTEROL, LIPID PHOSPHORUS AND C/P RATIOS OF RABBITS^a

Group ^b	Time in Weeks					
	0	4	8	12	16	20
I. Control						
Total Cholesterol ± S E	60.2 ± 1.7	38.6 ± 3.4	31.7 ± 2.1	34.1 ± 5.7	29.9 ± 3.6	43.4 ± 5.7
Lipid Phosphorus ± S E	6.06 ± 0.33	5.63 ± 0.33	4.78 ± 0.24	2.94 ± 0.30	3.25 ± 0.28	3.41 ± 0.23
C/P Ratio ± S E.	9.93 ± 0.22	6.86 ± 0.28	7.27 ± 0.29	11.6 ± 1.1	9.21 ± 0.69	12.6 ± 1.3
II. Cholesterol						
Total Cholesterol ± S E.	50.8 ± 5.5	119.7 ± 20.6	89.1 ± 13.8	97.5 ± 14.6	98.8 ± 18.1	100.4 ± 18.0
Lipid Phosphorus ± S E	5.94 ± 0.51	7.59 ± 0.67	6.94 ± 0.73	5.22 ± 0.63	4.22 ± 0.38	4.56 ± 0.40
C/P Ratio ± S E.	8.56 ± 0.27	15.7 ± 1.2	13.6 ± 0.87	18.7 ± 1.3	23.4 ± 2.3	22.3 ± .18
III. Nicotine						
Total Cholesterol ± S E.	70.5 ± 5.1	57.3 ± 6.2	47.3 ± 6.7	51.1 ± 6.3	58.2 ± 13.3	47.6 ± 8.1
Lipid Phosphorus ± S E.	6.41 ± 0.32	5.41 ± 0.21	6.19 ± 0.27	4.75 ± 0.21	5.25 ± 0.59	4.19 ± 0.43
C/P Ratio ± S E.	11.2 ± 0.71	10.9 ± 1.5	10.8 ± 0.97	12.2 ± 1.1	10.1 ± 0.91	9.29 ± 0.84
IV. Nicotine + Cholesterol						
Total Cholesterol ± S E.	61.7 ± 3.9	107.2 ± 15.1	112.3 ± 18.1	195.3 ± 31.7	194.7 ± 27.6	193.1 ± 32.8
Lipide Phosphorus ± S E	6.34 ± 0.36	7.38 ± 0.65	7.19 ± 0.82	7.13 ± 0.94	7.44 ± 0.69	8.38 ± 1.03
C/P Ratio ± S E	10.1 ± 0.32	13.7 ± 0.92	15.8 ± 2.1	26.1 ± 2.3	25.1 ± 2.0	22.1 ± 2.2

^a Total cholesterol and lipid phosphorus are expressed in mg. %.
^b Group I, Control, received stock diet, Group II, Cholesterol, 0.1% cholesterol in diet; Group III, Nicotine, 2.28 mg/Kg. nicotine daily in drinking water, and Group IV, Nicotine-Cholesterol, combined treatments of II and III.

terol group remained relatively constant at the eight- and twelve-week periods but began to increase at sixteen weeks.

The total cholesterol/lipid phosphorus (C/P) ratio is thought by some to be a more sensitive index of atherogenic susceptibility than is the plasma cholesterol level alone (29). Table I and Fig. 3 show that the control and nicotine C/P ratios did vary appreciably but that they are raised in both the cholesterol and nicotine-cholesterol groups. While the nicotine-cholesterol combination produced a faster rise in the ratios than cholesterol alone, the ratios of both groups were approximately equal at twenty weeks. Standard errors are included for each group mean in Table I, but because of inherent wide variations in the cholesterol and lipid phosphorus levels of rabbits (30) more sophisticated statistical approaches were employed. The data were first analyzed for variance by the hierarchic (31) and orthogonal (32) methods. Since significant differences were obtained between groups in both analyses, the two procedures were combined into a regression comparison analysis (33) to determine if the shape of the curves differ significantly from one another.

The *F* values and their significances are given in Table II. The hierarchic analysis of variance reveals significant differences between groups for cholesterol, lipid phosphorus, and the C/P ratios and also between rabbits within groups for cholesterol and lipid phosphorus, but not for the C/P ratios. It may be concluded that there was an overall real difference between the group values although not necessarily for all groups at all times.

The orthogonal analysis of variance also indicates significant differences between the groups and between the periods for the lipid phosphorus and the

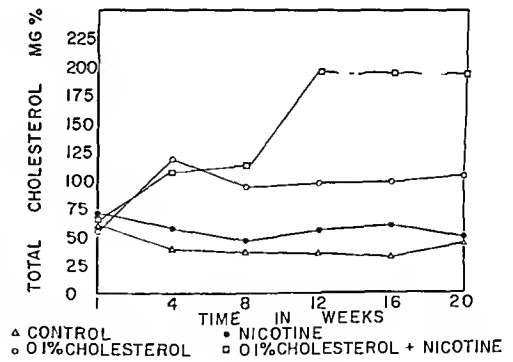


Figure 1.

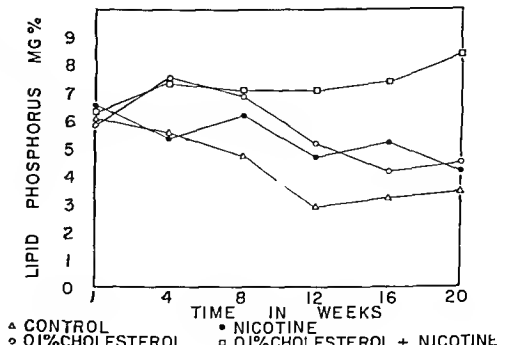


Figure 2.

TABLE II.—ANALYSIS OF VARIANCE

	Hierarchic F values	Orthogonal F values
Between groups		
Cholesterol	24.5 ^a	73.6 ^a
Lipid phosphorus	27.9 ^a	28.0 ^a
C/P ratio	45.1 ^a	88.1 ^a
Between rabbits within groups		
Cholesterol	3.16 ^a	..
Lipid phosphorus	3.02 ^a	..
C/P ratio	1.09 ^b	..
Between periods		
Cholesterol	..	1.72 ^b
Lipid phosphorus	..	8.19 ^a
C/P	..	28.61 ^a
Interaction		
Cholesterol	..	5.25 ^a
Lipid phosphorus	..	3.92 ^a
C/P ratio	..	8.43 ^a

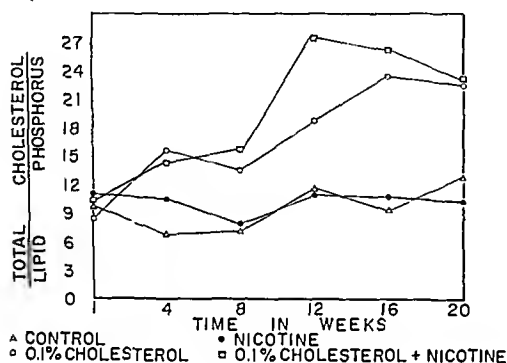
^a P < 0.001.^b Not significant.

Figure 3.

C/P ratios, but not between the periods for cholesterol. This is not to say that there are not significant differences between periods for cholesterol but only that the study as a whole shows no significant differences. This is clarified by the interaction between periods (P) and rabbits (R) which is significant

and demonstrates that real differences do exist between periods but not at all of the times. In other words, as can be seen in Fig. 1, the increases or decreases in plasma cholesterol for all groups did not always proceed in the same direction at one time and there was an overall cancelling effect. Therefore in order to determine whether the nicotine-cholesterol group, for example, had cholesterol values significantly different from those of the control the test period would have to be specified.

Since both the hierarchic and orthogonal analyses indicated significant differences between groups, the two procedures were combined into a regression comparison analysis (33) in order to show whether the shapes of the curves differed significantly for the four groups. The results of this analysis are listed in Table III. It can be seen that for several types of curves the differences are significant. This is especially true when they are compared as linear curves.

From the data and its statistical evaluation it may be concluded that while the plasma cholesterol level of the nicotine-cholesterol group was significantly increased by the administration of nicotine over that of the cholesterol group, the lipid phosphorus level of this group also increased. If the C/P ratio is used as an index of atherogenic susceptibility the hypercholesterolemia effect of nicotine is at least partially compensated for by the increase in lipid phosphorus. In order to assess any atherogenic tendency of nicotine it would therefore be necessary to determine the degree of pathological vascular involvement.

The experiment was originally designed to continue for twenty-eight rather than the twenty weeks as shown in the figures and tables. During the last eight weeks a number of animals died in the control, nicotine, and nicotine-cholesterol groups and while values were obtained with the survivors for two more periods they are not included in the data because little reliance could be placed upon the data from small groups. Nine animals were left in the control group, 12 in the cholesterol, five in the nicotine, and four in the nicotine-cholesterol.

At the end of twenty-eight weeks, tests for serum ascorbic acid, serum stability, and bromsulfalein retention were made and the gross aortic lesions, aortic cholesterol, body and liver weights determined

TABLE III.—REGRESSION ANALYSIS OF VARIANCE

Type of Curve	Cholesterol <i>F</i> values				Lipid Phosphorus <i>F</i> values				C/P Ratio <i>F</i> values			
	Control	Cholesterol	Nicotine	Nicotine-Cholesterol	Control	Cholesterol	Nicotine	Nicotine-Cholesterol	Control	Cholesterol	Nicotine	Nicotine-Cholesterol
Linear	18.6 ^c	9.38 ^b	16.0 ^c	52.4 ^c	125.5 ^c	29.8 ^c	26.7 ^c	8.13 ^b	17.3 ^c	209.0 ^c	^d	122.1 ^c
Quad-ratic	19.9 ^c	7.53 ^b	8.21 ^b	7.90 ^b	12.8 ^c	4.58 ^b	^d	^d	19.4 ^c	24.8 ^c	9.74 ^b	23.9 ^c
Cubic	^d	8.42 ^b	^d	^d	12.0 ^b	18.4 ^c	^d	^d	20.2 ^c	2.12 ^d	1.71 ^d	3.21 ^d
Quartic	^d	5.27 ^a	^d	^d	1.81 ^d	^d	^d	^d	15.9 ^c	25.3 ^c	^d	^d
Quintic	^d	2.48 ^d	7.71 ^c	3.70 ^d	6.11 ^a	^d	^d	^d	8.42 ^c	20.1 ^c	9.85 ^c	7.61 ^b

^a < 0.05; ^b P < 0.01; ^c P < 0.001.^d Not significant. Where values are not given whole numbers were not obtained.

TABLE IV—MEAN GROUP VALUES OF TERMINAL DETERMINATIONS

Group	Serum Ascorbic Acid, mg %	Serum Stability, % Trans mission	Aortic Total Cholesterol mg %	Brom sulfalein Retention, %	Body Weight, Gm	Liver Weight Gm
Control	0 73	101	11 1	2 2	3910	111
Cholesterol	0 81	78	16 8	6 7	3490	111
Nicotine	0 76	77	30 6	4 1	3561	113
Nicotine Cholesterol	0 57	96	18 9	5 6	3435	85

on the remaining animals. These values are listed in Table IV. While they cannot be considered to be meaningful because of the reduced number of animals, they are included for reference purposes. Simple analyses of variance were made for all these determinations but no significant differences could be demonstrated. The body weights are of some interest as the nicotine cholesterol group ate the least and therefore gained the slowest. Rabbits generally develop more arterial lesions if they are well fed and thriving than when they gain more slowly (30). In this instance the low weight group had the highest plasma cholesterol level.

These tests were performed in an attempt to determine the mechanism by which nicotine produced hypercholesterolemia. It was thought that the action of nicotine might be related to its effect on ascorbic acid as Bourquin (34) found that the level of ascorbic acid in whole blood was lowered as a result of smoking. This observation coupled with the gross and often complete deficiency of ascorbic acid in the arteries of atherosclerotic humans at autopsy and the fact that scurvy in guinea pigs results in rapidly developing atherosclerosis (35) makes the relationship of potential significance. The role of ascorbic acid in atherosclerosis is possibly a function of its control of the synthesis of cholesterol from active acetate (36) and (or) its importance in general vascular health. Serum stability was studied because of the observation by Ressler (25) that the serum of atherosclerotic individuals was relatively unstable, becoming turbid on the addition of certain metallic salts. Liver function was examined because the liver is the principal organ for cholesterol synthesis, turnover, and excretion. In diseases of the liver various alterations of lipid metabolism occur with associated derangements of plasma lipid patterns (37).

Gross examination of the aortas revealed plaques in five of the cholesterol group, three of the nicotine group and one of the nicotine cholesterol group. According to Katz's (27) system for the quantitative evaluation of atherosclerotic lesions they could be graded as class 1 and 2 involvement. This was then minimal atherosclerosis. Once again the inadequate group sizes made comparison inadvisable.

SUMMARY

1. Four groups of twelve rabbits per group were tested five times at four week intervals for total plasma cholesterol and plasma lipid phosphorus. The groups consisted of a control, 0.1% cholesterol diet, 2.28 mg/Kg/day nicotine alkaloid in the drinking water, and combined cholesterol and nicotine. At twenty eight weeks, stud-

ies were made of serum ascorbic acid, serum stability, and bromsulfalein retention. Animals were sacrificed and the aortas were examined for gross lesions and their cholesterol content determined. Terminal body and liver weights were also obtained.

2. Tests of significance demonstrated that the administration of nicotine in addition to a cholesterol containing diet caused significant increases in plasma cholesterol, lipid phosphorus and the C/P ratio of rabbits. The importance of the nicotine enhancement of hypercholesterolemia as an atherogenic stimulus may be negated by the concomitant rise in lipid phosphorus as reflected by the C/P ratios.

3. Significant differences could not be demonstrated in the serum ascorbic acid, serum stability, bromsulfalein retention, and aortic lesions possibly because of the mortality in some groups following the twenty-week period.

REFERENCES

- (1) Goetz R H, *Clin Proc*, 1, 190(1942)
- (2) Evans, W F, and Stewart, H J, *Am Heart J*, 26, 78(1943)
- (3) Weinroth L A, and Herzstein, J, *J Am Med Assoc*, 131, 205(1946)
- (4) Stewart, H J, *Med Clin North America*, 29, 599(1945)
- (5) Roth, G M, McDonald, J B, and Sheard, C, *J Am Med Assoc*, 125, 761(1946)
- (6) Lampson R S, *ibid*, 104, 1963(1935)
- (7) Wright, I S, *ibid*, 101, 439(1933)
- (8) Thienes, C H, and Butt, E M, *Am J Med Sci*, 195, 522(1938)
- (9) Weinroth L A, and Herzstein J, *J Am Med Assoc*, 131, 205(1946)
- (10) Hegghin, R and Keiser, G, *Schweiz Med Wochschr*, 85, 53(1955)
- (11) Sigler L H, *N Y State J Med*, 55, 3107(1955)
- (12) Hammond, E C, and Horn, D, *J Am Med Assoc*, 155, 1366(1954)
- (13) Russel, H I, Zohman B L, and Dorset, V J, *ibid*, 157, 568(1955)
- (14) Bargerion L M, Jr, Elmke, D, Gonulbol F, Castellanos A, Siegel, A, and Bing, R J, *Circulation*, 15, 251(1957)
- (15) Travell, J, Karp, D, and Rinzler, S H, *Fed Proc*, 16, 341(1957)
- (16) Ström A, and Jensen R, *Lancet*, 1, 126(1951)
- (17) Katz L N, and Stamler J, "Experimental Atherosclerosis" Charles C Thomas Springfield 1953 pp 20-24
- (18) Gertler M M, Garn, S M, and White, P D, *Circulation*, 2, 696(1950)
- (19) Keys A, *Bull Johns Hopkins Hosp*, 88, 473(1951)
- (20) Stamler J, and Katz, L N, *Circulation*, 2, 705(1950)
- (21) Johnston I M, *Lancet*, 243, 742(1942)
- (22) Koch F C, and Hanke M E, "Practical Methods in Biochemistry", 6th ed., Williams and Wilkins, Baltimore 1953 pp 220-222
- (23) Hawk P B, Osier B L, and Summerson W H, "Practical Physiological Chemistry", 13th ed., Blakiston New York 1954 pp 589-590
- (24) Lowry O H, Lopez, J A, and Bessy O A, *J Biol Chem*, 160, 609(1945)

- (25) Ressler, N., Boyl, A. J., and Kosai, M., *Am. J. Clin. Pathol.*, **24**, 194(1954).
 (26) Consolazio, C. F., Johnson, R. E., and Marek, E., "Metabolic Methods," C. V. Mosby, St. Louis, 1951, p. 412.
 (27) Horlick, L., and Katz, L. N., *Am. Heart J.*, **38**, 336 (1949).
 (28) Faber, M., and Lund, F., *Arch. Pathol.*, **48**, 351(1949).
 (29) Ahrens, E. H., Jr., *Bull. N. Y. Acad. Med.*, **26**, 151 (1950).
 (30) Davidson, J. D., *Am. J. Med.*, **11**, 736(1951).
 (31) Snedecor, G. W., "Statistical Methods," 4th ed., The Iowa State College Press, Ames, 1946, pp. 246-283.
 (32) *Ibid.*, pp. 237-244.
 (33) *Ibid.*, pp. 388-398.
 (34) Bourquin, A., and Musmanno, E., *Am. J. Digest. Diseases*, **20**, 75(1953).
 (35) Willis, G. C., and Fishman, S., *Can. Med. Assoc. J.*, **72**, 500(1955).
 (36) King, C. G., Bureh, H. E., Baker, R. R., and Salmon, L., *Federation Proc.*, **12**, 470(1953).
 (37) Peters, J. P., and Van Slyke, D. D., "Quantitative Chemical Chemistry," 2nd ed., Interpretations vol. I, Williams and Wilkins, Baltimore, 1946, pp. 156-158.

Effect of the pH of Buffered Paper on the R_f of Alkaloids*

By MICHAEL DEFFNER† and ALICE ISSIDORIDES-DEFFNER

The influence of pH upon the R_f and the nature of the buffer used for the pretreatment of the paper have been investigated for belladonna, strychnos, and opium alkaloids. R_f values at the same pH of the same buffer are correlated to their chemical structure. Variation of R_f with the pH of the buffer, variation of R_f for different buffers of the same pH, the influence of certain anions and cations, molarity of buffer, and the kind of paper used for chromatography are discussed.

ALKALOIDS and other basic substances with a dissociation constant of about 10^{-3} to 10^{-10} , chromatographed on untreated paper with neutral developing solvents, give diffuse elongated spots. This is due to their existence on the paper as ions and as undissociated molecules. These two forms have different R_f values and therefore give elongated spots (1-4).

Strong or very weak bases on the other hand, when chromatographed on untreated paper with neutral solvents, give sharp round spots because they exist in only one form either as ions or as undissociated molecules.

Alkaloids with a dissociation constant between 10^{-3} and 10^{-10} can be chromatographed on paper without "tailing" by addition of an acid to the developing solvent, or by pretreatment of the filter paper with neutral salts or buffer solutions of appropriate pH.

The addition of an acid to the developing solvent generally produces good chromatograms. The dissociation constant of the base determines the amount of the acid to be used. The addition of strong mineral acids or polycarboxylic acids to the developing solvent gives rise to the effect of "demixion" (Entmischung) (1). The ions of the slower moving strong acid remain behind the

solvent front forming a second front, the front of "demixion." If the alkaloids appear above or below the front of "demixion," this effect does not interfere in most cases with the identification and separation of the substances. However, if an alkaloid appears in the vicinity of the front of "demixion," double spots are formed which make the measurement of the R_f and separation of a mixture difficult. By increasing or decreasing the concentration of the acid, and thus moving the front of "demixion" above or below the spot of the alkaloid, the formation of double spots can be prevented.

The use of filter paper pretreated with neutral salts or buffer solutions also prevents "tailing." In the first case, the acid of the anion of the salt is added to the developing solvent; whereas in the second case, neutral developing solvents are used.

The R_f of an alkaloid chromatographed on paper pretreated with buffer solutions with *n*-butyl alcohol or isobutyl alcohol saturated with the same buffer solution as the developing solvent, depends on the pH and the nature of the buffer used (5). The lower the pH, the lower the R_f value of the alkaloid will be since the ion has lower R_f value than the undissociated base. Thus the R_f increases with increasing pH and becomes constant at a higher pH.

The purpose of this investigation was to determine the variation of the R_f for various alkaloids with different buffers. The alkaloids investigated were apatropine, atropine, hyoscyamine, scopolamine, brucine, strychnine, codeine, morphine, papaverine, and 1-benzyl-3-ethyl-6,7-dimethoxy-isoquinoline (isaverine, Merck).

EXPERIMENTAL

Apparatus and Reagents.—Beckman model G pH meter, *n*-Butyl alcohol reagent grade, chloroform

* Received January 8, 1957 from the Vitarine Greece Co., Athens, Greece.

† Associate Professor, University of Athens, Athens, Greece. The authors wish to thank Merck AG Darmstadt, and The Vitarine Co., Inc., New York, for supplying the compounds used in this study.

reagent grade, McIlvaine's buffer (6), citrate buffer (7), phosphate buffer 0.2 *M* U. S. P. XV, tartrate buffer 0.2 *M* (7), acetate buffer 0.2 *M* (7), lactate buffer 0.2 *M* (7), phthalate buffer U. S. P. XV, and Dragendorff's reagent as modified by Munier and Macheboeuf (1).

Preparation of Papers.—Strips of Whatman No. 2 paper, 4 x 30 cm., were immersed in different buffer solutions, air dried, sewn lengthwise with a white cotton thread in order of ascending pH, formed to fit the glass cylinder, and then placed therein.

Chromatography.—Glass cylinders (13 cm. diam., 45 cm. height) were used. The substances were applied with a micropipet as free bases in a chloroform solution (20 mcg. each time). The ascending method was used with *n*-butyl alcohol saturated with water as the developing solvent. A small beaker containing the water phase was placed at the bottom of the chromatographic jar, the hollow paper form was placed therein, and left six to fifteen hours for equilibration. The developing solvent was then added and the chromatograms were developed in the dark for six to eight hours (height: 20–25 cm.). The chromatograms were air dried and sprayed with Dragendorff's reagent and the *R_f* values were calculated, measuring the distance from the point of application to the leading edge of the spot. All chromatograms were run in duplicate and the *R_f* values, although varying with temperature, did not differ by more than 7%.

The pH of the buffer solutions was measured before and after immersion of the filter paper and after shaking the buffer solution with *n*-butyl alcohol. As Table I shows for citrate buffer, the pH remains unchanged after immersion of the paper, but increases slightly after shaking with *n*-butyl alcohol.

TABLE I.—pH OF BUFFER AFTER IMMERSION OF PAPER AND AFTER SHAKING WITH *n*-BUTYL ALCOHOL

Initial pH	pH After Immersion	pH After Shaking With <i>n</i> -Butyl Alcohol
1.22	1.22	1.32
2.20	2.15	2.30
3.20	3.20	3.30
4.30	4.30	4.40
5.40	5.40	5.50
6.00	5.95	6.10

Use of Whatman Nos. 1 and 4 and Schleicher and Schüll No. 2043b paper gave slightly different *R_f* values, but showed the same variation of *R_f* with the pH of the buffer solution used for the pretreatment of the paper. *R_f* values were always lower on Whatman No. 1 and Schleicher and Schüll No. 2043b than those obtained on Whatman No. 2 paper. On Whatman No. 4 paper they were highest. Whatman No. 1 paper sometimes gave "tailing," whereas Whatman No. 4 paper gave more diffuse spots. Changes in the molarity of the buffer did not bring about any appreciable changes in the *R_f* values, but the lower the molarity, the more elongated were the spots. The use of citrate buffer, prepared from secondary sodium citrate with the addition of HCl and NaOH, respectively (7), gave dif-

ferent *R_f* vs. pH graphs from those obtained when using citrate buffer prepared from citric acid by adding tertiary sodium citrate. Figure 1 shows that the *R_f* values up to about a pH of 5.0 are higher for the first buffer than those for the second. This is due to the influence of chlorine ions of HCl used for the preparation of the first buffer.

With pH's above 5.0 there is no influence of chlorine ions because NaOH instead of HCl was added to the buffer. The use of KOH instead of NaOH in the buffer gave higher *R_f* values which indicated some influence of the cations on the *R_f* values of the alkaloids. Figures 2, 3, 4, and 5 illustrate the dependency of the *R_f* values of the alkaloids on the pH for: citrate, McIlvaine's, phosphate, and tartrate buffers. The *R_f* values of the alkaloids at dif-

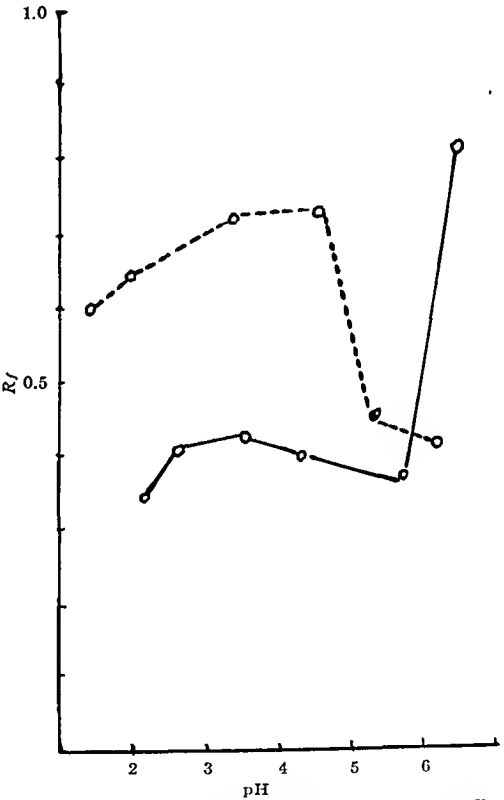
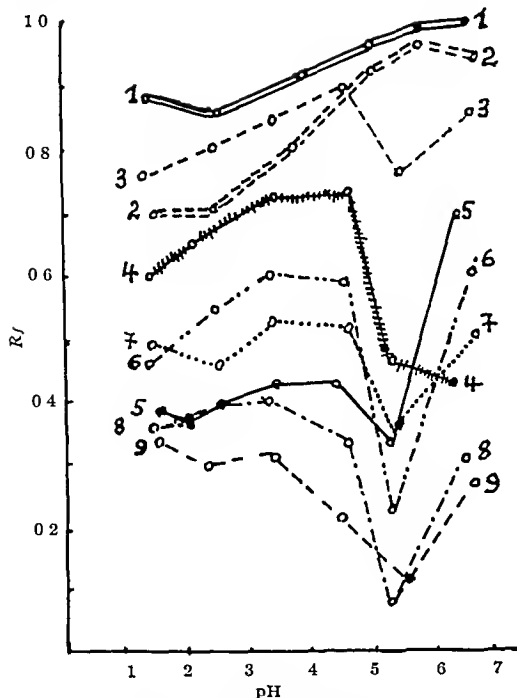


Fig. 1.—*R_f* vs. pH of atropine, ○- - -○, buffer prepared from secondary sodium citrate with the addition of HCl and NaOH; ○—○, buffer prepared from citric acid and tertiary sodium citrate.

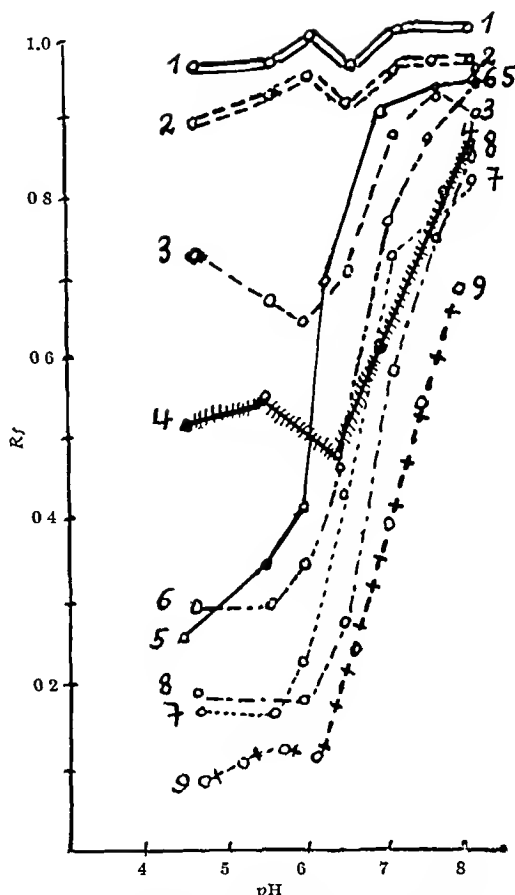
ferent pH's for acetate and lactate buffers are shown in Tables II and III. The *R_f* values of all alkaloids increased steadily with increasing pH for those two buffers. The *R_f* values for phthalate buffer are not shown because they caused only slight variation.

DISCUSSION

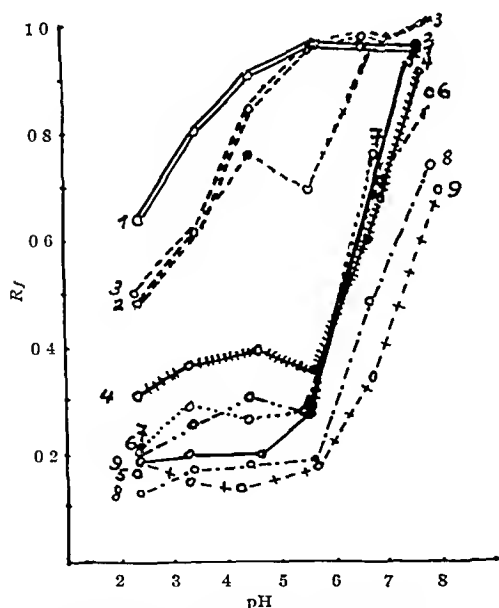
The *R_f* values of the compounds studied depended primarily on the partition between the stationary

Fig. 2.— R_f vs. pH of alkaloids, citrate buffer.

See Fig. 5 for legend of curves

Fig. 4.— R_f vs. pH of alkaloids, phosphate buffer.

See Fig. 5 for legend of curves

Fig. 3.— R_f vs. pH of alkaloids, McIlvaine's buffer.

See Fig. 5 for legend of curves

phase—adsorbed water—and the moving phase—organic solvent. The highly lipophilic compounds travelled as fast as the organic solvent having high R_f values. The hydrophilic ones having low R_f values remained close to the starting line. Thus the larger the alkyl group, the higher the R_f . Isoalkyl

compounds had lower R_f values than their *n*-alkyl analogs. Hydroxy compounds also had lower R_f values than their alkyl analogs. Cations and anions gave lower R_f values than the undissociated molecules, bases, and acids (8).

In the group of belladonna alkaloids, apatropine has atropic instead of tropic acid in the molecule, the $\text{CH}-\text{CH}_2\text{OH}$ group being substituted by a $\text{C}=\text{CH}_2$ group. Apatropine has therefore higher R_f values than atropine at all pH's of all buffers used. Scopolamine has, instead of the tropanol nucleus, an oscine nucleus with an oxygen bridge. Scopolamine has, at pH's above 5.50 and in acetate buffer, higher R_f values than atropine, but in all other buffers below pH 5.50, lower R_f values than atropine; probably because of the formation of oxonium ions at the lower pH levels.

In the group of strychnos alkaloids, brucine, a dimethoxystrychnine, has lower R_f values than strychnine.

The opium alkaloid codeine, is more lipophilic than morphine and therefore has higher R_f values. In the group of opium alkaloids with an isoquinoline nucleus, papaverine, because of the two additional $-\text{OCH}_3$ groups and the lack of the $-\text{C}_2\text{H}_5$ group, has lower R_f values than isaverine (1-benzyl-3-ethyl-6,7-dimethoxy-isoquinoline).

TABLE II— R_f VALUES OF ALKALOIDS AT DIFFERENT pH'S FOR ACETATE BUFFER

pH	C	M	P	I	ST	B	AT	SC	AP ^a
3.3	0.84	0.64	0.86	1.00	0.74	0.60	0.60	0.84	0.76
3.7	0.89	0.70	0.88	1.00	0.80	0.64	0.74	0.90	0.87
4.3	0.94	0.72	0.91	1.00	0.88	0.76	0.82	0.94	0.94
4.9	0.97	0.82	0.92	1.00	0.95	0.85	0.94	0.99	0.97
5.6	0.97	0.80	1.00	1.00	0.95	0.90	0.96	1.00	1.00
6.1	0.95		1.00	1.00	0.95	0.86	0.98	1.00	1.00

TABLE III— R_f VALUES OF ALKALOIDS AT DIFFERENT pH'S FOR LACTATE BUFFER

pH	C	M	P	I	ST	B	AT	SC	AP ^a
2.5	0.42	0.32	0.63	0.72	0.42	0.40	0.48	0.32	0.62
3.0	0.46	0.39	0.66	0.76	0.48	0.42	0.54	0.38	0.67
3.4	0.54	0.39	0.72	0.82	0.60	0.50	0.60	0.46	0.76
4.2	0.62	0.45	0.82	0.85	0.72	0.62	0.74	0.56	0.86
4.8	0.64	0.40	0.84	0.86	0.76	0.68	0.82	0.66	0.90
5.2	0.64	0.44	0.84	0.78	0.78	0.70	0.82	0.67	0.90

^a C, Codeine, M, Morphine, P, Papaverine, I, Isaverine, ST, Strychnine, B, Brucine, AT, Atropine, SC, Scopolamine, AP, Apoptropine

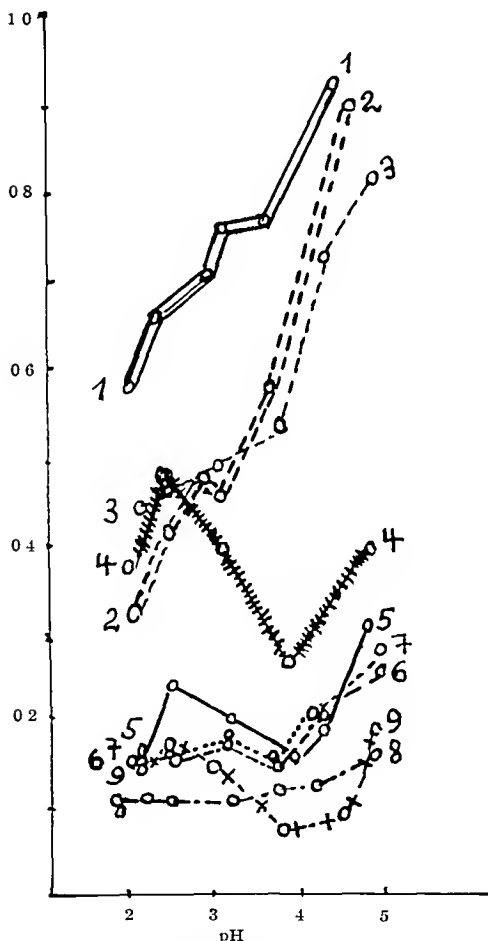


Fig. 5.— R_f vs. pH of alkaloids, tartrate buffer. Figures 2, 3, 4, and 5: 1---1 isavrine, 2---2 papaverine, 3---3 apoatropine, 4---4 atropine, 5---5 scopolamine, 6---6 strychnine, 7---7 codeine, 8---8 brucine, 9---9 morphine

l-Hyoseyamine, in spite of its difference in the R_f values in certain buffers, especially tartrate, cannot be separated from atropine, its *dl*-isomer.

All alkaloids investigated, with the exception of papaverine and isaverine, belong to the group of alkaloids with dissociation constants from 10^{-3} to 10^{-7} and show a variation of R_f with pH, the R_f generally increasing with increasing pH. Papaverine and isaverine, which belong to the group of alkaloids with dissociation constants from 10^{-7} to 10^{-10} , being weaker bases, show this variation at lower pH levels. The R_f values of each alkaloid show differences at the same pH levels depending on the buffer used. These differences can not be explained by the difference in ionic strength because they exist even in buffers of approximately the same ionic strength, but they may also be due to differences in the water solubility of the solvent (9) or a chemical interaction between the anion of the buffer and the alkaloid. In the case of buffers of di- and poly-carboxylic acids, the R_f does not always increase by increasing the pH and the graphs R_f vs. pH show minima at certain pH levels (Figs. 2, 3, and 5). This may be due to the fact that di- and, in general, poly-carboxylic acids exist in different ionic forms depending on the pH.

The graphs R_f vs. pH are characteristic of a compound or a group of compounds and may be used for identification. They may also be used to find the optimum pH for the separation of compounds in a mixture.

REFERENCES

- (1) Loiseleur, J., "Techniques de Laboratoire," vol. 2, 2nd ed., Masson et Cie, Paris, 1954, p. 372.
- (2) "Chromatographie," Merck AG Darmstadt, p. 53.
- (3) Schultz, O. E., and Strauss, D., *Arzneimittel-Forsch.*, 5, 342(1955).
- (4) Block, R. J., Durrum, E. L., and Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press Inc., New York, 1955, p. 244.
- (5) Brindle, H., Carlsson, J. E., and Woodhead, H. B., *Quart. J. Pharm. and Pharmacol.*, 3, 793(1951).
- (6) McIlvaine, T. C., *J. Biol. Chem.*, 49, 183(1921).
- (7) Bertho, A., and Grassmann, W., "Biochemisches Praktikum," Walter de Gruyter Co., Berlin, 1930, p. 13-15.
- (8) Fieser, L. F., "Experiments in Organic Chemistry," 3rd ed., D. C. Heath and Co., Boston, 1955, pp. 130-142.
- (9) McFarren, E. F., *Anal. Chem.*, 23, 168(1951).

The Synthesis and Pharmacology of N-Substituted Aminoalkyl Esters of Chlorophenyl Carbamic Acid*

By ELIAS EPSTEIN and DANIEL KAMINSKY

Dialkylamino and morpholino alkyl esters of *ortho*, *meta*, and *para*-chlorophenyl carbamic acids were prepared and tested for their local anesthetic efficiency (ratio of potency to toxicity) and for irritation. Most of these compounds proved to be too irritating for clinical use but a few were sufficiently low in irritating properties to warrant further investigation.

THE GREATEST NUMBER of local anesthetics introduced for use by the medical and dental professions are esters of benzoic or substituted benzoic acids. Nevertheless, the property of producing local anesthesia is not limited to this type of structure, but is found in amides, amino-alcohol ethers, esters, and a wide variety of organic compounds.

Several of the esters of phenylcarbamic acids have been prepared and their anesthetic action noted (1-19). It was of interest to us to prepare the N-substituted aminoethyl and aminoisopropyl esters of the three isomeric chlorophenyl car-

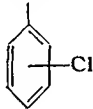

bamates and to screen them for their relative anesthetic efficiency (ratio of relative potency to relative toxicity).

Table I lists the melting points of the anesthetic bases together with the melting points, analyses, and molecular weight determinations of the hydrochloride salts. Table II lists the relative toxicities, potencies, irritation, and efficiencies of these compounds.

EXPERIMENTAL

The general method of synthesis was the well-known one of reacting an isocyanate with an active

TABLE I—N-SUBSTITUTED AMINOALKYL ESTERS OF CHLOROPHENYL CARBAMIC ACID

(a) $\text{NH}-\text{COOCH}_2-\text{CH}_2\text{R}$									
									
No	Cl Position	R	m p base	m p HCl	Formula	Per Cent Theory	Ionic Cl Found	Molecular Theory	Weight Found
1	<i>o</i>	Dimethylamino	Oil	166-168	$\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2\text{Cl}_2$	12 70	12 64	279	274
2	<i>o</i>	Diethylamino	Oil	160-163	$\text{C}_{13}\text{H}_{20}\text{O}_2\text{N}_2\text{Cl}_2$	11 55	11 49	307	305
3	<i>o</i>	Morpholino	73-75	193-196	$\text{C}_{13}\text{H}_{18}\text{O}_3\text{N}_2\text{Cl}_2$	11 04	10 91	321	319
4	<i>o</i>	Di-isopropylamino	Oil	136-141	$\text{C}_{15}\text{H}_{24}\text{O}_2\text{N}_2\text{Cl}_2$	10 58	10 44	335	331
5	<i>o</i>	Dibutylamino	Oil	138-145	$\text{C}_{17}\text{H}_{26}\text{O}_2\text{N}_2\text{Cl}_2$	9 76	9 58	363	359
6	<i>m</i>	Dimethylamino	74-77	169-171	$\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2\text{Cl}_2$	12 70	12 49	279	273
7	<i>m</i>	Diethylamino	Oil	140-144	$\text{C}_{13}\text{H}_{20}\text{O}_2\text{N}_2\text{Cl}_2$	11 55	11 38	307	305
8	<i>m</i>	Morpholino	119-121	222-224	$\text{C}_{13}\text{H}_{18}\text{O}_3\text{N}_2\text{Cl}_2$	11 04	10 97	321	320
9	<i>m</i>	Di-isopropylamino	Oil	164-167	$\text{C}_{15}\text{H}_{24}\text{O}_2\text{N}_2\text{Cl}_2$	10 58	10 42	335	332
10	<i>m</i>	Dibutylamino	Oil	168-173	$\text{C}_{17}\text{H}_{26}\text{O}_2\text{N}_2\text{Cl}_2$	9 76	9 98	363	366
11	<i>p</i>	Dimethylamino	56-58	173-176	$\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2\text{Cl}_2$	12 70	12 89	279	280
12	<i>p</i>	Diethylamino	Oil	175-177	$\text{C}_{13}\text{H}_{20}\text{O}_2\text{N}_2\text{Cl}_2$	11 55	11 29	307	306
13	<i>p</i>	Morpholino	166-169	229-230	$\text{C}_{13}\text{H}_{18}\text{O}_3\text{N}_2\text{Cl}_2$	11 04	10 89	321	318
14	<i>p</i>	Di-isopropylamino	Oil	181-183	$\text{C}_{15}\text{H}_{24}\text{O}_2\text{N}_2\text{Cl}_2$	10 58	10 43	335	331
15	<i>p</i>	Dibutylamino	Oil	160-161	$\text{C}_{17}\text{H}_{26}\text{O}_2\text{N}_2\text{Cl}_2$	9 76	9 91	363	364
(b) $\text{NH}-\text{COOCH}(\text{CH}_3)-\text{CH}_2\text{R}$									
									
16	<i>o</i>	Dimethylamino	Oil	179-182	$\text{C}_{12}\text{H}_{18}\text{O}_2\text{N}_2\text{Cl}_2$	12 10	11 99	293	292
17	<i>p</i>	Dimethylamino	124-126	189-191	$\text{C}_{12}\text{H}_{18}\text{O}_2\text{N}_2\text{Cl}_2$	12 10	11 98	293	290

* Received September 23, 1957 from the Research Laboratories of the Novocel Chemical Mfg. Co., Inc., Brooklyn, N. Y.

We are indebted to Ann Hartman for assistance in the pharmacological testing of these compounds.

hydrogen, in this case the hydrogen bonded to the oxygen atom of the aminoalcohol. The chlorophenyl isocyanates react with the aminoalcohols in an inert solvent such as benzene to form the carba-

TABLE II.—PHARMACOLOGY OF N-SUBSTITUTED AMINOALKYL ESTERS OF CHLOROPHENYL CARBAMIC ACIDS

No.	Relative Potency				Relative Toxicity			Irritation		Relative Efficiency ^b
	Rabbit Eye ^a	Guinea Pig Wheel ^b	Guinea Pig Sciatic Nerve ^c No. Epin	Guinea Pig With Epin	White Mouse ^b Intra-peritoneal	White Mouse ^b Subcutaneous	Rabbit Intra-venous	Eye	Skin	
1	0 5	0 2	1 7	1 2	1 1	0 9	3	0	0	0 1
2	0 3	3 7	5	1 1	1 5	2	>4	0	0	2.5
3	0 3	0 08	0 3	0 8	0 8	0 8	0.7	++	+	0.1
4	1	1 1	2	3	3	2	>4	0	+	0 4
5	2	2 4	1 5	4	0 5	0 5	>4	++	+	4.8
6	c	0 9	1 6	2	0 7	0 8	0 8	++	+	1.3
7	1	1 5	1 4	2	1 1	2	>4	++	+	1.4
8	0 2	0 5	1 0	0 5	0 5	0 6	<0 6	+	+	1.0
9	1	1 5	3	4	1 5	3	>4	+	+	1.0
10	2	4 1	1 5	3	0 9	0 5		+	+	4.5
11	c	0 6	0 9	1 7	1 6	3		++	++	0 4
12	0 2	2 1	1 3	1 7	0 9	1 0	1 6	+	0	2 3
13	0 2	0 2	0 3	<0 1	0 5	0 7	0 7	0	+	0 4
14	0 3	0 4	2	1 1	0 6	1 0	1 3	0	0	0.7
15	4	2 4	d	d	d	d	d	0 ^e	d	
16	1	1 1	4	3	0 8	0.8	1 1	+	+	1.4
17	c	1 1	1 7	0 8	0 7	0.9		++	++	1.6

^a Cocaine equals 1 at 0.2%^b Procaine equals 1^c Too irritating for test^d Too insoluble for test.^e No apparent irritation at 0.2%

mates in good yield. Several of the hydrochlorides were insoluble in water and were isolated by adding dilute hydrochloric acid to the benzene reaction mixture and filtering. The anesthetic compounds were purified by recrystallization of the hydrochloride salts from isopropyl alcohol.

The *ortho*, *meta*, and *para*-chlorophenyl isocyanates were commercially available. The aminoalcohols, also commercially available, were carefully fractionated before use.

Diethylaminoethyl Ester of *m*-Chlorophenyl Carbamic Acid.—To a solution of 16.9 Gm. (0.11 mole) *m*-chlorophenyl isocyanate in 50 ml. of dry benzene was added 11.7 Gm. (0.1 mole) of diethylaminoethanol dissolved in 50 ml. of dry benzene. The mixture was allowed to stand at room temperature for twenty-four hours and then heated to the boiling point. After cooling to room temperature, the mixture was filtered and the filtrate extracted with two 250-ml. portions of 15% hydrochloric acid. The acid extract was made alkaline with excess concentrated ammonium hydroxide. The anesthetic base was extracted with two 200-ml. portions of ether, dried over anhydrous sodium sulfate, and bone-charred. The ether solution was acidified with anhydrous hydrochloric acid. The precipitate on recrystallization from 99% isopropyl alcohol yielded 29.3 Gm. (96% of theory) diethylaminoethyl ester of *m*-chlorophenyl carbamic acid, as white crystals, m. p. 140–142°.

PHARMACOLOGY

The anesthetic potency of these compounds was determined by three methods: topical application to the rabbit cornea; blocking the sciatic nerve of the intact guinea pig; and by wheal tests on the back of the guinea pig. The toxicity of these compounds was determined subcutaneously and intraperitoneally on white mice and intravenously on rabbits.

Topical Anesthetic Potency.—This method, originated by Koller and described by Hirschfelder and Bieter (20), was modified as follows: 0.25 ml. of

solution was instilled into the conjunctival sac of the rabbit's eye and the lower lid was slightly raised to allow the solution to bathe the cornea of the eye for thirty seconds. The presence of anesthesia was determined by the absence of the wink reflex when the cornea of the eye was stimulated by a long hair obtained from the rabbit's maxilla. The relative topical anesthetic potency was determined by extrapolating results at different concentrations to the concentration which would give a duration of local anesthesia equal to a 1% cocaine hydrochloride solution.

Conductive Anesthetic Potency.—This method, originally introduced by Shackell (21), involves blocking the sciatic nerve in the guinea pig under conditions not too different from those in clinical practice. Two-tenths of a ml. of solution was injected into the bony furrow between the trochanter of the femur and the vertebral column where the sciatic nerve emerges from the spinal column. The loss of sensation at the calf of the leg was determined by pinching lightly with a tweezer. The relative potency was determined by extrapolating the concentration which gave anesthesia for the same duration as one per cent procaine solution. This was repeated, using solutions containing 1:100,000 epinephrine.

Infiltration Anesthetic Potency.—The method used consisted of raising wheals on the guinea pig back with 0.1 ml. of different concentrations of the compound, waiting fifteen minutes, and then determining the presence or absence of anesthesia by touching the center of the wheal with the point of a needle. The relative potency was determined by comparing the lowest concentration of the anesthetic that will produce anesthesia 50% of the time on the guinea pig and comparing this value to that obtained with procaine hydrochloride.

Subcutaneous Toxicity on White Mice.—The relative subcutaneous toxicity of each compound was determined by comparing the LD₅₀ with that obtained for procaine hydrochloride. The tests were run at three critical dose levels, with three animals at each level, using a minimum of nine

animals per compound. The critical dose is one near or at the LD₅₀. The numerical value of the LD₅₀ was calculated by the method of summation of the animals living and dying at each dose level and determining the point where the two curves would cross (22, 23). White male Swiss mice of approximately 15 Gm. in weight were carefully injected subcutaneously with a 27 gauge needle over the abdominal area with a 2-4% solution of the compound. If no wheal formed or if leakage was observed during or just after the injection, the results of that test were discarded. Toxic symptoms developed in a few minutes and lasted up to several hours. The animals were observed for a twenty-four hour period and those animals alive and appearing normal were considered to have survived the test.

Intraperitoneal Toxicity on White Mice.—The relative intraperitoneal toxicities were determined by comparing the LD₅₀ of these compounds with that of procaine hydrochloride in the same manner as for the subcutaneous injection. Toxic symptoms developed sooner and lasted for a shorter time than with the subcutaneous injection. The same number of animals and dose levels were used as for the determination of the subcutaneous toxicity.

Intravenous Toxicity on Rabbits.—The relative intravenous toxicity on rabbits was determined for the most part only on those compounds which were not too irritating. A minimum of four rabbits per compound was used in this determination. The intravenous dose was diluted with normal saline solution to a volume equal to 2 ml. per Kg. of body weight and injected over a period of one minute into the marginal ear vein of the rabbit. Toxic symptoms usually developed immediately after the injection was completed. Those animals alive after twenty-four hours were considered to have survived.

Irritation Test on the Rabbit Eye.—The eye of the rabbit was closely examined for evidence of irritation, one hour and twenty-four hours after instillation of a 1% solution of the compound. The results are recorded in Table II as follows: (a) Little or no irritation: 0, no or slight hyperemia of the conjunctiva. (b) Moderate irritation: +, moderate hyperemia of the conjunctiva with the eyelids remaining open and no exudate present. (c) Severe irritation: ++, severe hyperemia of the conjunctiva with the eyelids closed with or without the presence of exudate.

Trypan Blue Irritation Test.—All of the compounds were tested for irritation on the rabbit skin. A minimum of two rabbits were used for each compound. The method consisted of raising a wheal on the abdomen of the rabbit and subsequently injecting a trypan blue solution intravenously in the ear. The method is essentially that of Weatherby (24). The results were recorded in Table II, using the same classification as for the corneal irritation test.

Relative Anesthetic Efficiencies.—Relative anesthetic efficiencies were determined by dividing the relative potency, as determined by the guinea pig wheal test, by the relative intraperitoneal toxicities on white mice. The guinea pig wheal test for anesthetic potency of commercially used anesthetics gives a close correlation to clinical findings. The relative toxicity obtained with intraperitoneal injection on white mice was used, since it represents

a value between that of the intravenous toxicity, where the drug is absorbed rapidly; and that of the subcutaneous toxicity, where the drug is absorbed slowly.

DISCUSSION AND SUMMARY

Seventeen N-substituted aminoalkyl esters of chlorophenyl carbamic acid were prepared and tested pharmacologically. The relative potency, toxicity, irritation, and anesthetic efficiency were determined. The greater number of this group of compounds were irritating. The toxicities were in the same order of magnitude as other commonly used anesthetics.

The following general correlation between molecular structure and anesthetic efficiency can be made.

1. The diethyl and dibutyl aminoethyl esters are more efficient than the dimethyl, diisopropyl, and morpholino aminoethyl esters.

2. N-Substituted aminoisopropyl esters are more efficient than the corresponding N-substituted aminoethyl esters.

3. The position of the chlorine atom on the benzene ring does not impart any clear cut differences in effectiveness of the compound.

Several of the more promising anesthetic compounds are being tested clinically.

REFERENCES

- (1) Fromberz, H., *Arch. expil. Pathol. Pharmacol.*, **76**, 257(1914).
- (2) Rider, T. H., *J. Pharmacol. Exptl. Therap.*, **39**, 457(1930).
- (3) Rider, T. H., *J. Am. Chem. Soc.*, **52**, 2115, 2583(1930).
- (4) Horne, W. H., Cox, R., Shriner, R. L., *ibid.*, **55**, 3435(1933).
- (5) Cook, E. S., and Rider, T. H., *ibid.*, **58**, 1079(1936).
- (6) Rider, T. H., and Cook, E. S., *J. Pharmacol. Exptl. Therap.*, **64**, 1(1938).
- (7) Donleavy, J. J., and English, J., Jr., *J. Am. Chem. Soc.*, **62**, 2965(1940).
- (8) Cope, A. C., and Hancock, E. M., *ibid.*, **66**, 1448(1944).
- (9) Ramsey, H., and Haag, H. B., *J. Pharmacol. Exptl. Therap.*, **70**, 1(1942).
- (10) Hirschfelder, A. D., and Bieter, R. N., *J. Am. Chem. Soc.*, **61**, 1(1939).
- (11) Rau, G. C., and Westfall, B. A., *J. Pharmacol. Exptl. Therap.*, **99**, 421(1950).
- (12) Sekera, A., Hruby, J., Vrba, C., and Lebduška, J., *Chem. listy*, **44**, 275(1950); through *Chem. Abstr.*, **46**, 3122f(1951).
- (13) Bandelin, F. J., and Tuschoff, J. V., *THIS JOURNAL*, **40**, 302(1951).
- (14) Sekera, A., Jakubec, I., Kral, J., and Vrba, C., *Chem. listy*, **46**, 762(1952); through *Chem. Abstr.*, **47**, 12302(1953).
- (15) Sekera, A., Hruby, J., Vrba, C., and Lebduška, J., *Czechoslov. farm.*, **1**, 554(1952); through *Chem. Abstr.*, **47**, 9792i(1953).
- (16) Vrba, C., Lebduška, J., and Sekera, A., *ibid.*, **1**, 554(1952); through *Chem. Abstr.*, **47**, 12635h(1953).
- (17) Rothstein, R., Gley, P., de Boislaumont, P., Binovic, K., Stoven, O., and Delor, J., *Compt. rend.*, **239**, 284(1954).
- (18) Hutton, K., *J. Org. Chem.*, **20**, 808, 855(1955).
- (19) Sekera, A., Borovansky, A., Jakubec, I., Palat, K., and Vrba, C., *Czechoslov. farm.*, **5**, 388(1955); through *Chem. Abstr.*, **51**, 8669(1956).
- (20) Hirschfelder, A. D., and Bieter, R. N., *Physiol. Rev.*, **12**, 190(1932).
- (21) Shackell, L. F., *Anesthesia & Analgesia*, **14**, 120(1935).
- (22) Irwin, J. O., and Cheeseman, E. A., *J. Hyg.*, **39**, 192(1938).
- (23) Bliss, C. I., *Quart. J. Pharm. and Pharmacol.*, **11**, 192(1938).
- (24) Weatherby, J. H., *J. Lab. and Clin. Med.*, **25**, 1199(1940).

Determination of the Minimal Carcinogenic Dose₅₀ of Methylcholanthrene on Mouse Epidermis*

By RONALD F. GAUTIERI and DAVID E. MANN, Jr.

The minimal carcinogenic dose₅₀ (MCD₅₀) of methylcholanthrene necessary to induce squamous cell carcinomas on the epidermis of CF-1 mice was found to be 504 micrograms applied through 21 biweekly applications of 0.02 milliliter of a 0.12 per cent solution of methylcholanthrene. The MCD₅₀ was confirmed by a second experiment which yielded similar results.

SINCE TANNENBAUM and Silverstone (1) have conclusively demonstrated that the effect of an inhibitory procedure on tumor incidence with moderate doses of a carcinogen was obliterated by massive doses of the same agent, it becomes imperative to define the minimal dose of a carcinogen that is necessary to induce growths in a given percentage of animals in a definite time interval. Methylcholanthrene has been shown to be both consistent and potent in its ability to produce epidermal tumors when painted on the backs of mice (2, 3, 4). Similarly, this agent is valuable, when used as the strong carcinogen, in studying additive and inhibitory effects in conjunction with weaker carcinogens (2, 3, 4). If a satisfactory standardized dose/response relationship could be established for this agent, experimental procedures involving the inhibition or the enhancement of tumor growth would be more clearly evaluated with a better understanding of the mechanisms underlying the cancerization process.

Cramer and Stowell (5) have devised a technique that involves a quantitative estimation of the amount of methylcholanthrene painted on the interscapular region of mice with each stroke of a number 4 camel's hair brush. Berenblum and Schoental (6) have stated that if this carcinogen is applied for a considerable length of time and the effective concentration on the epidermis is sufficient, methylcholanthrene will induce cancers in 100 per cent of the mice exposed to it. The injectable tumor dose₅₀ of methylcholanthrene has been found to be 0.021 mg. by Bryan and Shimkin (7). Berenblum (8) has estimated that the topical tumor doses of various carcinogens appear to be approximately 50 times greater than the corresponding injectable doses.

It is the purpose of this paper to define a MCD₅₀ for methylcholanthrene on mouse epidermis, utilizing various concentrations of this carcinogen and employing newer techniques for the controlled administration to the sheared, interscapular region of albino mice.

MATERIALS AND METHODS

The experimental plan was conducted in two separate parts for the purpose of evaluating the effectiveness and the reproducibility of the procedures in producing dose/response relationships that are relatively constant with the concomitant verification of the MCD₅₀.

In part I, 120 CF-1 albino mice (60 males and 60 females), were divided into 12 groups of equal sex; in part II, 90 CF-1 albino mice (45 males and 45 females), were divided into 9 groups of equal sex. Each group was labeled according to the concentration of methylcholanthrene which subsequently was to be applied to it. Each mouse approximately twelve weeks old, was placed in an individual metal cage constructed with a wire mesh front and bottom. Excreta pans were arranged to permit coprophagy to take place. The diet consisted of Purina Laboratory Chow and tap water, both given *ad libitum*. Food was placed in ample amounts on the floor of each cage and water was obtained through a glass drinking tube that was attached to a six-ounce bottle. The diet was supplemented once a week with lettuce and carrots for two successive weeks, followed by another two-week interval in which the supplement consisted of bread soaked in milk mixed with 2 ml. per quart of Vi-Penta drops.

Care was taken to eliminate all drafts and the temperature was maintained at 25° throughout the experiment. The cages were rotated before a window to standardize the amount of visible light to which the mice were exposed. This was done to conform with the observation of Morton, *et al.* (9), that light can influence tumor growth.

Two hundred and fifty milliliters of a 0.15% stock solution of methylcholanthrene in acetone were prepared. Aliquot portions of this stock solution were measured with syringe-adapted transfer pipets and added to the proper amount of acetone to make 30 ml. of solution ranging in concentration from 0.04 to 0.15% in increments of 0.01%. All measurements were made at room temperature.

In order to facilitate the handling of the methylcholanthrene solutions, ampuls were made and stored in a refrigerator at 0-5° until used.

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For a quantitative study of the amount of carcinogen necessary to induce tumors a more exact method of application than the method of Cramer and Stowell (5) is required. In this experiment, micro-pipets, with a capacity of 0.2 ml., were employed. With these pipets, 0.02 ml. could be precisely measured and applied to the interscapular region.

EXPERIMENTAL

Heavy Neoprene gloves were used to protect the hands while handling the mice and apparatus. The mice were always grasped by the tail with long forceps and transferred to the left hand and held at the base of the tail by the thumb and index finger. In this position they were then placed under the micro-pipet for application of the carcinogen to the interscapular space.

Each mouse received 0.02 ml. of its designated concentration of methylcholanthrene solution twice a week applied to the interscapular region, which was first sheared with an electric clipper and then once each following week with sharp barber scissors.

Upon the appearance of the first growth, each animal was then carefully examined four times a week with a model 701-A Stanley magnifying glass. At the termination of both parts of the experiment, all tumors were drawn and recorded on special data sheets that gave all of the necessary information concerning each mouse that showed evidence of tumor inducement. Also, several mice with typical growths were chloroformed and the tumors excised and immersed in Bouin's fixative for histological examination.

At the termination of both phases of the experiment all pertinent data were assembled and placed in four tables. Only those tumors which measured at least one dimension (width *versus* height) of 1 mm. or greater were recorded. Growths which did not conform to this criterion were extremely difficult to identify as epitheliomas. Table I (Part I) and Table III (Part II) show the chronological induction time and the quantity in micrograms of methylcholanthrene applied to the mice through the exact day on which a tumor was recorded according to the above-mentioned specifications. Table II (Part I) and Table IV (Part II) contain the complete data with respect to the separate groups at the termination of each phase of the experiment and relate

TABLE I.—MICROGRAM QUANTITIES OF METHYLCHOLANTHRENE APPLIED UNTIL ONSET OF TUMORS

No. of Mice	Sex	Concn., % M.C.A.	No. of Applications	Total Admin., mcg.	Induction Time, Days
90	F	0.12	14	336	49
96	F	0.13	16	416	55
119	F	0.15	16	480	55
118	F	0.15	17	510	58
51	M	0.09	18	324	62
70	F	0.10	18	360	62
74	M	0.11	18	396	62
88	F	0.12	18	432	62
109	F	0.14	18	504	62
102	M	0.14	18	504	63
100	F	0.13	19	494	64
84	M	0.12	19	456	65
106	F	0.14	19	532	65
46	F	0.08	20	320	69
79	F	0.11	20	440	69
83	M	0.12	20	480	69
86	F	0.12	20	480	69
93	M	0.13	20	520	69
99	F	0.13	20	520	69
110	F	0.14	20	560	69
2	M	0.04	21	168	72
19	F	0.05	21	210	72
31	M	0.07	21	294	72
36	F	0.07	21	294	72
87	F	0.12	21	504	72
89	F	0.12	21	504	72
117	F	0.15	21	630	72

the total quantity of methylcholanthrene administered in mcg. with tumor incidence per group during the 10-week duration of each phase of the experiment.

Epilation was noticed in most animals beginning about four to five days after the first application of all of the various solutions of methylcholanthrene. The epilation became more and more pronounced for about 18 days, at which time many of the animals showed evidence of practically complete epilation of the interscapular region and its vicinity to which the carcinogen had spread by capillarity. For the next few days, this nearly complete epilation persisted and was then followed by another period during which there occurred slight stimulation of hair growth for one week. The mice which exhibited marked epilation then underwent a resurgence of hair growth which left these animals with

TABLE II.—INCIDENCE OF TUMOR INDUCTION WITH BIWEEKLY APPLICATIONS OF VARIOUS CONCENTRATIONS OF METHYLCHOLANTHRENE TO MICE FOR 10.4 WEEKS

Concn of Methylchol., %	Total Admin., mcg.	Effective Total, Mice/Group	No. Tumors/Group	Incidence of Tumors, %/Group	Sex	
					M	F
0.04	168	10	1	10.0	1	
0.05	210	8	1	12.5		1
0.06	252	10	0	0.0		
0.07	294	10	2	20.0	1	1
0.08	336	8	1	12.5		1
0.09	378	7	1	14.3	1	
0.10	420	8	1	12.5		1
0.11	462	10	2	20.0	1	1
0.12	504	9	7	77.8	2	5
0.13	546	10	4	40.0	1	3
0.14	588	9	4	44.4	1	3
0.15	630	9	3	33.3		3

TABLE III.—MICROGRAM QUANTITIES OF METHYLCHOLANTHRENE APPLIED UNTIL ONSET OF TUMORS

No. of Mice	Sex	Concn., % M.C.A.	No. of Applications	Total Admin., mcg.	Induction Time, Days
81	M	0.12	15	360	53
117	F	0.15	15	450	53
88	F	0.12	15	360	54
43	M	0.08	17	272	58
110	F	0.14	17	476	60
105	M	0.14	18	504	63
90	F	0.12	19	456	64
65	M	0.10	19	380	67
79	F	0.11	19	418	67
86	F	0.12	19	456	67
101	M	0.14	19	532	67
114	M	0.15	19	570	67
113	M	0.15	20	600	69
36	F	0.07	21	294	72
40	F	0.07	21	294	72
61	M	0.10	21	420	72
78	F	0.11	21	462	72
85	M	0.12	21	504	72
87	F	0.12	21	504	72

Histopathological examination of several typical tumors revealed that these growths were true cancers of the type that are produced on the epidermis by methylcholanthrene, *vis.*, squamous cell carcinomas.

DISCUSSION

As shown in Tables II and IV, the results were similar in each phase (Part I and Part II) of the experiment. At the termination of each phase, the animals which received biweekly applications (on Tuesdays and Thursdays) of a 0.12% solution of methylcholanthrene in acetone gave evidence of at least 50% carcinogenesis. Each member of this group received a total of 504 mcg. of methylcholanthrene. Therefore, this total dose administered through 21 applications of 0.02 ml. of a 0.12% methylcholanthrene/acetone solution may be designated the MCD₅₀ necessary to induce tumors on the epidermis of CF-1 mice. The tumors so induced measured at least one dimension (width *versus* height) of 1 mm. or greater.

The minimal carcinogenic dose₅₀ appears to be the

TABLE IV.—INCIDENCE OF TUMOR INDUCTION WITH BIWEEKLY APPLICATIONS OF VARIOUS CONCENTRATIONS OF METHYLCHOLANTHRENE TO MICE FOR 10.4 WEEKS

Concn of Methylchol., %	Total Admin., mcg.	Effective Total, Mice/Group	No. Tumors/Group	Incidence of Tumors %/Group	Sex	
					M	F
0.07	294	10	2	20.0		2
0.08	336	10	1	10.0	1	
0.09	378	10	0	0.0		
0.10	420	10	2	20.0	2	
0.11	462	10	2	20.0		2
0.12	504	10	6	60.0	2	4
0.13	546	9	0	0.0		
0.14	588	10	3	30.0	2	1
0.15	630	10	3	30.0	2	1

longer hair in the interscapular region than on any other area of their bodies.

Nine days after the first application of the respective solutions of methylcholanthrene, slight vasodilation of subcutaneous blood vessels was observed with the naked eye. This phenomenon persisted in most animals throughout the experiment, while some of the mice had small subcutaneous hemorrhages that often broke the surface of the epidermis.

Of the 210 animals employed in both phases of the experiment, only 19 died. Fourteen males and five females succumbed due to causes other than those of the experiment. The mice that died showed no signs of injured epidermis or hemorrhage in the interscapular region. The animals that died became progressively sicker and succumbed within a five to nine-day period. Only one mouse died after the appearance of a tumor.

Among the 42 control animals (21 males and 21 females), seven deaths were recorded, all of them males. At no time did a spontaneous tumor appear in any of the control animals. Also, by breeding several members from the control group, it was possible to check the appearance of spontaneous growths in the progeny. Through many successive generations there was no evidence of such spontaneity.

optimal dose for tumor formation since both larger and smaller doses within the range of this experiment exerted a delay in the onset of carcinogenesis. It is extremely difficult to present an adequate explanation of this phenomenon since a paucity of fundamental knowledge regarding the intracellular action of methylcholanthrene exists in the literature. One might hypothesize in view of these results that a threshold exists for a maximal carcinogenic response. Doses which fail to attain this threshold perhaps are unable to cause an interaction with the intracellular receptors, a condition which may trigger off the transformation to malignancy. On the other hand, doses which surpass the threshold may inactivate the receptors by being present in greater amounts, thereby retarding the cancerization process.

The procedure outlined herein has been shown to yield results that are both constant and reproducible. This technique may be employed in future studies to determine various dose/response relationships for all of the other hydrocarbon carcinogens, with the possible clarification of some of the basic principles which concern the cancerization process.

There were 29 females and 17 males which developed measurable tumors during the course of the entire experiment. Therefore, it appears that

methyleholanthrene causes a higher tumor incidence in female mice under these conditions. Females may respond more readily due to the higher mitotic activity and thickness of the epidermis in the anterior dorsal region just prior to estrus (10).

The epilation and subcutaneous vasodilation caused by methyleholanthrene are in accord with the findings of Cramer and Stowell (5). The resurgence of hair growth in most animals is probably the stage in which hypertrophy and hyperplasia of the epidermal tissues occur. This, therefore, may be regarded as the period during which there is a transformation from the normal stage to one of malignancy, later developing into squamous cell carcinoma.

REFERENCES

- (1) Tannenbaum, A., and Silverstone, H., *Cancer Research*, **7**, 567 (1947).
- (2) Wirtmann, W. B., Shubik, P., Hill, W. T., Reeb, B. B., Stanger, W. D., and Riegel, D., *ibid.*, **11**, 301 (1951).
- (3) Lucassagne, A., Bun-Hoi, N. P., and Rudnik, G., *Brit. J. Exptl. Pathol.*, **26**, 5 (1955).
- (4) Steiner, P. E., and Pnlk, H. L., *Cancer Research*, **11**, 56 (1951).
- (5) Cramer, W., and Stowell, R. E., *J. Natl. Cancer Inst.*, **2**, 369 (1952).
- (6) Berenblum, I., and Schoental, R., *Cancer Research*, **7**, 390 (1947).
- (7) Bryan, W., and Shimkin, M. B., *J. Natl. Cancer Inst.*, **3**, 503 (1943).
- (8) Berenblum, I., "Advances in Cancer Research," New York Academic Press, Inc., Vol. 11, 1951, p. 129.
- (9) Morton, J. J., Mider, G. B., Luce-Clausen, E. M., and Mahoney, E. B., *Cancer Research*, **11**, 559 (1951).
- (10) Bullough, W. S., *J. Endocrinol.*, **6**, 340 (1950).

Polyphosphoric Acid in the Bischler-Napieralski Reaction*

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The use of polyphosphoric acid in syntheses of 1-substituted 3,4-dihydroisoquinolines from N-acyl phenethylamines has been investigated, and a method of synthesis resulting in greatly improved yields has been developed. Two new heterocyclic bases have been prepared, and several previously unreported derivatives have been listed. A heterocyclic base not previously prepared from an N-acyl phenethylamine has been synthesized.

THE UTILITY of polyphosphoric acid as a dehydrating agent and as a cyclizing agent has been demonstrated repeatedly in the recent literature. The apparent versatility of this reagent suggested its use as the cyclizing agent for N-acyl phenethylamines in Bischler-Napieralski syntheses of 1-substituted 3,4-dihydroisoquinolines. Leonard and Boyer (1) prepared 1-methyl-3,4-dihydroisoquinoline in 20% yield from N-(β -phenethyl) cyanoacetamide, using a commercial polyphosphoric acid in the ratio of five grams to one gram of the amide. Snyder and Werber (2) cyclized acetyl phenethylamine by heating it with ten times its weight of a commercial polyphosphoric acid; they obtained a 23% yield of 1-methyl 3,4-dihydroisoquinoline. These workers similarly prepared 3,4-dihydroisoquinoline in 31% yield. Pratt, Riee, and Luckenbaugh (3) reported a 79% yield of crude 3,4-dihydroisoquinoline in a polyphosphoric acid condensation of N-formyl phenethylamine. Murakoshi (4) prepared 1-benzyl-3,4-dihydroisoquinoline in 20% yield by treating N-phenylacetyl phenethylamine with a mixture of phosphorus oxychloride and extemporaneously pre-

pared polyphosphoric acid. Hey and Lobo (5) obtained a 39% yield of 1-benzyl-3,4-dihydroisoquinoline on refluxing N-phenylacetyl phenethylamine with polyphosphoric acid in xylene solution.

Literature reports concerning the general applicability of polyphosphoric acid to Bischler-Napieralski cyclizations of N-acyl phenethylamines are incomplete; therefore, a study was undertaken to evaluate the use of the reagent in the preparation of a series of 1-substituted 3,4-dihydroisoquinolines. In all reactions reported here, freshly prepared polyphosphoric acid was used in the ratio of 330 grams per 0.1 mole of amide; this was roughly equivalent to 10 to 15 grams of polyphosphoric acid per gram of the amide. Yields of 3,4-dihydroisoquinolines were, in every instance where comparison was possible, superior to those reported previously in the literature.

1-Isopropyl- and 1-isobutyl-3,4-dihydroisoquinoline have not been listed in the literature prior to this paper. In connection with other studies, a sample of 1-isobutyl isoquinoline was needed; this was also a new compound, and it was prepared by aromatization of the corresponding 3,4-dihydroisoquinoline. The identity of the aromatized compound was confirmed by comparison with a sample prepared by an entirely different method (6). The methiodides of

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all of the heterocyclic bases reported in this paper have not been previously listed in the literature

EXPERIMENTAL

N-Acyl Phenethylamines.—Several methods were employed. (a) Condensation of the acid chloride with an equimolecular quantity of phenethylamine in the presence of an equivalent portion of pyridine; (b) the Schotten-Baumann Method, (c) refluxing the acid chloride with an equimolecular quantity of the amine in benzene solution, (d) refluxing equimolecular amounts of the acid and the amine. See Table I

Polyphosphoric Acid.—One hundred and seventy-five grams of phosphorus pentoxide was weighed into a round-bottom flask, and to it was added in small amounts and with agitation 100 ml of 85% orthophosphoric acid. The mixture was agitated for about one-half hour, until all solid matter had dissolved, it was then used immediately in the cyclization reactions

3,4-Dihydroisoquinolines.—One tenth mole of the N-acyl phenethylamine was added with thorough

mixing to the freshly prepared polyphosphoric acid. The resulting homogeneous mixture, protected by a calcium chloride drying tube, was heated in an oil bath for three hours at a bath temperature of 200–205°

While still hot, the viscous dark liquid was poured over an excess of ice contained in a large round-bottom flask, and the reaction flask was rinsed with small portions of water, which were added to the ice-water mixture. A concentrated solution of sodium hydroxide was then added with agitation and cooling until the contents of the flask were strongly alkaline to litmus. This mixture was then steam distilled, and the distillate was extracted three times with benzene. The benzene extracts were combined and dried over anhydrous magnesium sulfate, filtered, and the benzene was removed on a steam bath by use of a water aspirator. The dark residue was then distilled under reduced pressure. 1-Phenyl- and 1-benzyl-3,4-dihydroisoquinoline were not steam distilled; rather, the basified reaction mixtures were extracted several times with benzene to separate the products. See Table II.

1-Isobutyl Isoquinoline.—Ten grams of 1-iso-

TABLE I—ACYL PHENETHYLAMINES

"R"	b p or m p °	mm	Yield, %	Method of Prepn d	Formula	Analysis ^d
H	146–152	0.8	67			
CH ₃	128–135	1	55	a		
C ₂ H ₅	150–155	0.8	65	a		
n-C ₃ H ₇	155–160	0.8	78	a		
iso-C ₃ H ₇	80–81 ^a		45	a	C ₁₂ H ₁₇ NO	N Calcd, 7.34, Found, 7.15
n-C ₄ H ₉	147–152	0.15	62	a		
iso-C ₄ H ₉	57–59 ^a		71.5	a	C ₁₃ H ₁₉ NO	N Calcd, 6.82, Found, 6.79
n-C ₆ H ₁₁	154–156 32–34 ^a	0.03	40	a	C ₁₄ H ₂₁ NO	N Calcd, 6.40, Found, 6.58
C ₆ H ₅	115–116 ^b		90	b		
Benzyl	87–89 ^c		75	c		
Cyclohexyl	97–98 ^c		39	c		

^a Recrystallized from aqueous ethanol

^b Recrystallized from 95% ethanol

^c Recrystallized from benzene

^d Compounds for which an analysis is given are those not previously reported in the literature. Analyses for nitrogen by Drs. Weiler and Strauss, Oxford, England

^e All melting points uncorrected

TABLE II—3,4-DIHYDROISOQUINOLINES

"R"	b p or m p °	mm	Yield, %	n _D ²⁰	Formula	Analysis ^b
H	75–77	1	75	1.5815 (24°)		
Picrate	173–175					
Methiodide	125–126				C ₁₀ H ₁₂ NI	N Calcd, 5.13, Found, 4.90

TABLE II —(continued)

CH ₃	70-76	1	82	1 5738 (20°)		
Picrate Methiodide	188-192 200-202				C ₁₁ H ₁₄ NI	N Calcd, 4 88, Found, 4 70
C ₂ H ₅	79-86	1	77 2	1 5632 (25°)		
Picrate Methiodide	195-197 113-115				C ₁₇ H ₁₇ NI	N Calcd, 4 64, Found, 4 35
<i>n</i> C ₃ H ₇	112-113	1	71 8	1 5532 (22°)		
Picrate Methiodide	162-165 ^c 132-134				C ₁₃ H ₁₈ NI	N Calcd, 4 44, Found, 4 29
^{iso} C ₃ H	85-95	1 5	62 3	1 5453 (22°)		
Picrate	171-172				C ₁₈ H ₁₈ N ₄ O ₇	N Calcd, 13 95, Found, 13 96
Methiodide	202-205				C ₁₃ H ₁₈ NI	N Calcd, 4 44, Found, 4 33
<i>n</i> -C ₄ H ₉	87-93	0 15	82 8	1 5443 (25°)		
Picrate Methiodide	149-150 112-113				C ₁₄ H ₂₀ NI	N Calcd, 4 26, Found, 4 32
^{iso} -C ₄ H ₉	69-72	1	68	1 5448 (20°)	C ₁₃ H ₁₇ N	N, Calcd, 7 48 Found, 7 34
HCl	126-129				C ₁₃ H ₁₈ NCI	Cl Calcd, 15 84, Found, 15 92
Picrate	150-153				C ₁₉ H ₂₀ N ₄ O ₇	N Calcd, 13 52, Found, 13 40
Methiodide	108-110				C ₁₄ H ₂₀ NI	N Calcd, 4 26, Found, 4 08
<i>n</i> C ₅ H ₁₁ ^d	102-106	0 15	67 5	1 5383 (25°)		
Picrate Methiodide	111-112 70-71				C ₁₅ H ₂₀ NI	N Calcd, 4 08, Found, 4 18
Cyclohexyl	114-120	0 15	70	1 5568 (24°)		
Picrate Methiodide	169-170 181-183				C ₁₆ H ₂₀ NI	N Calcd, 3 95, Found, 4 08
C ₆ H ₅	128-138	1	79	1 6295 (25°)		
Picrate Methiodide	173-175 188-190				C ₁₆ H ₁₆ NI	N Calcd, 4 01, Found 3 81
Benzyl	132-140	0 01	84	1 6228 (25°)		
Picrate Methiodide	180-182 205-207				C ₁₇ H ₁₈ NI	N Calcd, 3 87, Found, 3 83

^a All melting points uncorrected^b Compounds for which an analysis is given are those not previously reported in the literature Analyses for nitrogen by Clark Microanalytical Laboratory Urbana Ill and Drs Weiler and Strauss Oxford England^c Spath Berger and Kuntara *Ber* 63, 134(1930) reported m p 173-174^d Prepared by Broderick and Short *J Chem Soc* 1951, 1343 from N 2 phenethyl benzamidine chloride Synthesis from the *reyl* phenethylamine not previously reported

butyl-3,4 dihydroisoquinoline was heated with an approximately equal weight of freshly prepared Raney nickel until the mixture began to boil. It was then cooled, filtered, and the filter paper and its contents were thoroughly washed with ether, which was added to the filtrate. The ether was removed from the filtrate on a steam bath and the dark residue was distilled under reduced pressure, b p 129-131° (7.5 mm). Yield, 3.24 Gm (32.8%) of a light yellow liquid that soon darkened, *n*_D 1.5539

Picrate, recrystallized from absolute ethanol, m p (uncorrected) 168-171°

REFERENCES

- (1) Leonard N J, and Boyer, J H, *J Am Chem Soc*, 72, 2950(1950)
- (2) Snyder, H R and Werber, F X, *ibid*, 72, 2962 (1950)
- (3) Pratt, F F, Rice, R G, and Luckenbaugh, R W *ibid*, 79, 1212(1957)
- (4) Murakoshi, I *J Pharm Soc Japan* 76, 1139(1956)
- (5) Hey, D H and Lobo, I C *J Chem Soc* 1954, 2246
- (6) Cannon, J G and Webster, G L, *THIS JOURNAL* 46, 416(1957)

Vitamin B₁₂ in the Presence of Vitamin B₁ and Niacinamide in Aqueous Combinations*

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New facts about the physical and chemical stability of B-complex aqueous solutions containing vitamin B₁₂ are reported. Conditions concerning the preparation of such combinations are conclusively established. Limitations of artificial aging tests are given.

IN A RECENT PAPER (1) several statements were made on the stability of vitamin B₁₂ in the presence of other B complex components in aqueous solutions and, in addition to the problem of chemical stability, some physical changes were pointed out. Through further investigations it was possible to confirm more definitely the indispensable conditions to stabilize, chemically and physically, aqueous combinations of vitamins B₁, B₁₂ and niacinamide

COMMENTS

Concentration.—Gunsberg, *et al* (2, 3), were the first to point out the importance of the relative concentrations of vitamin B₁₂ and other components. They worked with solutions which had a higher proportion between the vitamin B₁ and vitamin B₁₂ concentrations than 500 to 1, while Macek and Feller (4, 5) made their studies using such proportions

Searches conducted to establish the limits of these proportions, previously considered by us to be ideal at 60 to 1 (1), led us to work with solutions containing vitamin B₁ and vitamin B₁₂ in proportions as high as 10,000 to 1 (Table I, solutions 1 to 8). We found that in proportions of 120 and 500 to 1 the losses were negligible at room temperature after a short period of time and after a three week exposure to 37° they were 12.2–15.4% and 23.8–26.7% respectively (Table I, solutions 3 and 5). For the proportions of 5,000 to 1 and 10,000 to 1 we found losses of 70.1% and more than 95% after a three week exposure to 37° (Table I, solutions 7 and 8). Thus, the higher the proportions the greater the inactivation of vitamin B₁₂.

These results agree with those of Macek and Feller (4, 5) and Gunsberg, *et al* (2, 3), and allowed us to conclude that considerably higher proportions than 120 to 1 between vitamin B₁ and vitamin B₁₂ are not recommended for aqueous combinations of vitamin B₁ with vitamin B₁₂ in the presence of other B-complex components

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The microbiological assays given in this paper were made in the laboratory of the Biochemical Department, College of Pharmacy, University of São Paulo, Brazil, by Miss Aurora Leal, Assistant to Professor Henrique Tastaldi, to whom the authors are gratefully indebted.

Purity of Components.—All raw materials must be of the highest purity. Vitamin B₁, particularly, must be carefully tested "for parenteral use" as described in the experimental part. By using two pure U. S. P. B₁ vitamins, purchased on the market from different sources and both being "for parenteral use" but one of them not specifically retested just before processing, results as demonstrated by solutions number 4 and 6 compared to number 5 and 7 (Table I) may occur.

pH Value.—Our previous experiences (1) and the observations of Macek and Feller (4, 5) seem to indicate that the ideal range of pH is from 3.0 to 4.0. We obtained the best results at pH 3.3.

Product-Processing.—We sterilized the solutions by filtration through Chamberland L5 micro porous porcelain candles. In addition to all the care previously described (1), the individual filtration of each vitamin is very important and is essential for vitamin B₁ and niacinamide.

"Liquid Volume to Air Volume" Ratio.—It was defined by us that fading of B complex solutions containing vitamin B₁₂ is a neat characteristic of vitamin B₁₂ inactivation, but its darkening, as a rule, does not prove the same thing (1). Only negligible differences were observed on physico-chemical (6) and microbiological (7) vitamin B₁₂ assay results in a lot of two samples, one of which was darkened deeply and the other with slight physical changes. Both samples were prepared from one solution and tested under the same conditions (Table I, solutions 1 and 3). The darkening, however, was the greatest trouble we had.

We observed a definite influence of the amount of air present over the solutions in the ampuls on the physical stability regarding discoloration. Different ratios were used between the liquid volume filled in the ampuls and the air volume (or air space) present in the sealed ampuls. We found that the darkening of high concentrated B complex aqueous combinations containing vitamin B₁₂ increases in the presence of decreasing amounts of oxygen (air). Actually, stable aqueous combinations of vitamin B₁₂ and other B-complex components, from a physical as well as chemical point of view, were possible only when a "liquid volume to air volume" ratio of 1.73 to 1.00 in the ampuls was observed (Table I, solutions 1 to 5).

In the course of our work, in order to emphasize the above mentioned points, we had the opportunity of proving again, by parallel microbiological assays, the applicability of Marsh and Kuzel's vitamin B₁₂ assay method (6) in stability studies of aqueous combinations of vitamin B₁₂ and other ionizable components (Table I, solutions 1 and 3). Limitations

decomposition becomes greater with the higher the temperature and longer the time of exposure

EXPERIMENTAL

All recommendations for raw materials and apparatus to be used should be exactly the same as previously described (1). The assays of the raw materials were performed according to the U S P standards. An additional qualitative test for vitamin B₁ must be accomplished: only raw materials giving a completely clear and colorless 30% solution in double distilled water observed in a flint glass cylinder of at least 15 cm diam must be used. For the individual filtration of the different components in an all glass or glass lined apparatus, a special assembly for vacuum filtration with a lot of micro porous porcelain candles was designed.

Each candle, free from heavy metal traces (8), was previously washed with 1,000 cc of 0.1 N hydrochloric acid, subsequently with 1,000 cc of hot double distilled water, and finally with cold distilled water until all chloride ions were removed. After heat sterilization of the apparatus, the filtration of the different vitamin solutions was made by suction. One candle must be used for vitamin B₁ and another for naeiamide. Vitamin B₆ and vitamin B₁₂ can be filtered through the same candle. The pH of the different vitamin solutions was previously ascertained by partial neutralization with an experimentally determined quantity of analytical reagent grade sodium bicarbonate so that the final solutions to be subdivided in the ampuls has the expected value. First we prepared and filtered a 30% solution of the vitamin B₁, adjusted to pH 3.3. We then filtered through another candle a 20% solution of the naeiamide, adjusted to pH 3.3 with analytical reagent grade hydrochloric acid. Finally we filtered a 10% solution of the vitamin B₆ containing the vitamin B₁₂, adjusted to pH 3.3. After every filtration, each candle was washed with double distilled water until desired volume.

The filling of the ampuls must be made so that the ratio between the liquid volume and the air volume present in the sealed ampuls is 1.73 to 1.00.

For the microbiological assay of vitamin B₁₂, the U S P method, using *Lactobacillus leichmanni*, was applied (7). The other analyses were made by the same methods as previously indicated (1).

The final sterility of all solutions was bacteriologically confirmed.

RESULTS AND DISCUSSION

It was possible to obtain chemically stable aqueous combinations containing vitamins B₁, B₆, and naeiamide if a certain pH value and a maximum proportion of 120 to 1 between vitamin B₁ and vitamin B₆ are observed. Rapid inactivation of vitamin B₁₂ occurs in solutions which have a proportion of 5,000 to 1 or 10,000 to 1, even when refined pharmaceutical methods are applied for the preparation of these parenteral forms.

The darkening is considerably increased at higher pHs [4.0 and 4.5, solutions number 9 and 12 described in Table II of the previous paper (1)] and at high temperature levels (37° and 45°, Table I, solutions 3 and 5). Different air volumes present over the solutions in the ampuls also influence definitely the physical stability of these combina-

tions. Using liquid volume to air volume ratios from 2.76 to 1.00 until 1.73 to 1.00 we obtained different degrees of darkening, from deep discolorations to no color changes. Characteristic results are demonstrated by Table I, solutions 1, 2, 3, and 5. Lower ratios than 1.73 to 1.00, i. e., presence of greater excesses of air in the sealed ampuls may cause a white precipitate. At a liquid volume to air volume ratio of 1.73 to 1.00 neither darkening nor precipitation were observed (Table I, solution 2 after 6 mrt and then three weeks 37° exposure, solution 3 after three weeks 45° exposure and after 9 mrt, and solution 5 after three weeks 37° exposure).

The results regarding discoloration of B complex aqueous combinations containing vitamin B₁₂ are contrary to the well-known observations of darkening of B complex aqueous combinations without vitamin B₁₂ as a result from thiamine decomposition influenced by the air present over the solutions. Even at a pH 3.3, vitamin B₁, vitamin B₆, and naeiamide aqueous combinations darken in the presence of large air volume in the ampuls, while in the presence of small quantities of air they are perfectly stable (9). Nevertheless, these combinations in the presence of vitamin B₁₂ and in the concentrations we worked, gave us the results previously discussed.

The chemical problems related to these physical changes are still being studied.

Summarizing, the important factors to obtain stable aqueous combinations on these vitamins are (a) the relative vitamin B₁ concentration present in combinations of vitamin B₁₂ with other B complex components, (b) the pH value, (c) the "liquid volume to air volume" ratio in the ampuls or vials.

The general interest to perform artificial aging tests at temperatures of 100° and 120° in this type of solution seems to disappear in view of the achieved high vitamin B₁₂ losses. Tests performed for a few weeks at only 37°, 40°, and 45° seem to give more conclusive stability results.

The individual filtration of the different vitamins is necessary to improve the chemical and physical stability of these solutions. The decomposition of vitamin B₁ in the presence of naeiamide is increased by the candles, in spite of the previous washing treatment where at least all calcium ions were removed.

Vitamin B₆ does not interfere in the stability of these combinations if the indicated product processing rules are strictly observed.

SUMMARY

1 The importance of the relative proportion between vitamin B₁ and vitamin B₁₂ was pointed out in view of the stability of aqueous combinations containing vitamin B₁, vitamin B₁₂, and naeiamide.

2 The darkening of B complex solutions containing vitamin B₁₂ in high concentrations was avoided by increasing the air content over the solutions in the ampuls.

3 Special emphasis was given on the importance of the processing rules concerning the preparation of the discussed parenteral solutions.

4. Good stability of vitamin B₁₂ was obtained even in presence of 60 mg./cc. of vitamin B₁ and 55 mg./cc. of niacinamide.

REFERENCES

- (1) Gambier, A. S., and Rahn, E. P. G., *THIS JOURNAL*, 46, 134(1957).
- (2) Blitz, M., Eigen, E., and Gunsberg, E., *ibid.*, 43, 651(1954).

- (3) Blitz, M., Eigen, E., and Gunsberg, E., *ibid.*, 45, 803(1956).
- (4) Macek, T. J., and Feller, B. A., *ibid.*, 44, 254(1955).
- (5) Feller, B. A., and Macek, T. J., *ibid.*, 44, 662(1955).
- (6) Marsb, M. M., and Kuzel, N. R., *Ind. Eng. Chem., Anal. Ed.*, 23, 1773(1951).
- (7) "United States Pharmacopeia," 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 885.
- (8) Taub, A., and Paikoff, S., *THIS JOURNAL*, 41, 248(1952).
- (9) Gambier, A. S., and Rahn, E. P. G., "Stability of injectable B-complex aqueous combinations without vitamin B₁₂," paper presented to the Section of Manufacturing Pharmacy, Fourth Pan-American Congress of Pharmacy and Biochemistry, Washington, D. C., Nov. 5, 1957.

A Chromatographic-Spectrophotometric Method for the Separation and Determination of Colchicine*

By STANISLAUS J. SMOLENSKI, FRANK A. CRANE, and RALPH F. VOIGT

An improved procedure for the extraction, separation, and purification of colchicine from plant tissue is presented. The method makes use of a simple separation and purification of colchicine by adsorption on two different chromatographic columns, eluting these with different solvents and determining the purified alkaloid colorimetrically.

A NUMBER OF METHODS have been devised for the extraction, separation, purification, and assay of colchicine. The extraction of the alkaloid has been carried out by making use of water (1-3), alcohol (4-8), wax and paraffin-wax (for the removal of resin) (4, 5), and a Soxhlet apparatus (8, 9).

Separation of colchicine from corm and seed tissue has been carried out by using chloroform (1-10). Determinations were made (a) gravimetrically, after extracting with methyl alcohol, shaking out the colchicine from chloroform in a separatory funnel (1, 6, 7); (b) polarigraphically, after extraction with water (3); and (c) colorimetrically (10, 11). Purification has been carried out on a chromatographic column, (12) making use of chloroform as the eluent.

In this study the alkaloid was extracted by a one per cent solution of ethyl alcohol in ethyl acetate after the removal of fixed oils by petroleum ether in a Soxhlet apparatus. This method makes use of a simple separation and purification of colchicine by adsorption on two successive chromatographic columns, eluting these with different solvents and determining the purified alkaloid colorimetrically. For this purpose Struve's color reaction (13) was adapted and the developed color was stable for at least three hours.

According to Struve (13), on addition of concentrated nitric acid colchicine changes from a yellow powder to a violet solution, which further changes, in a few minutes to brown-red; and then upon the addition of water, to yellow. On the further addition of sodium hydroxide T. S. the color becomes orange-red. This color test was applied in this laboratory to 28 other alkaloids or their salts, and none of them gave the identical reaction to colchicine in all three phases, i. e., with nitric acid, water, and sodium hydroxide T. S. For this reason Struve's color reaction was used throughout this work during the separation and purification of colchicine for testing the completion of extraction, and was further adapted for the quantitative spectrophotometric assay.

EXPERIMENTAL

Reagents Used.—1. Solvents used in extraction were petroleum-benzin, anhydrous ethyl acetate containing 1% ethyl alcohol, and benzene C. P. 2. Chromatograms were prepared in glass chromatographic tubes using (a) alumina-oxide-Alcoa, mesh 80-200, grade F-20 (not reacted before use), protected at both ends by a thin layer of Hyflo-Suprcel (Johns-Manville), and (b) silicic acid gel (ppt. SiO₂·H₂O) analytical reagent, Mallinckrodt, immersed in chloroform. 3. For eluting alumina column chromatograms, three successive solutions were used: (a) Chloroform U. S. P. XIV, (b) 4% solution of methyl alcohol U. S. P. XIV in Chloroform U. S. P. XIV, (c) 10% solution of methyl alcohol U. S. P. XIV in Chloroform U. S. P. XIV.

For eluting silica gel chromatograms, five successive solvents were employed: (a) chloroform, U. S. P. XIV, (b) 1% solution of methyl alcohol in chloroform U. S. P. XIV, (c) 2% solution of methyl

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alcohol in chloroform, (d) 10% solution of methyl alcohol in chloroform, and (e) 20% solution of methyl alcohol in chloroform. 4. Solutions used for the color reaction determining the presence of colchicine included concentrated nitric acid (reagent grade), distilled water, and sodium hydroxide T. S. This test was applied wherever colchicine was to be detected.

Extraction.—A representative sample of 10.0 Gm. of colchicum seed, reduced to a coarse powder (20-mesh) is extracted for four hours with 100 ml. of petroleum benzin in a Soxhlet apparatus. This solvent containing fats and resins is removed and the flask is replaced by one containing 100 ml. of anhydrous ethyl acetate with 1 ml. of ethyl alcohol. The powder is macerated for eight to twelve hours followed by continuous extraction for eight hours.

At the end of this period, 2 ml. of the solvent are removed from the Soxhlet extractor, evaporated to dryness, and tested for completeness of extraction of colchicine. The crude extract containing colchicine is concentrated to dryness by distillation. Five ml. of methyl alcohol are added to the residue and evaporated to dryness to remove all traces of ethyl acetate. The dried residue is taken up in 3 ml. of methyl alcohol, which is poured into a separatory funnel attached to a chromatographic column. The alcohol insoluble fraction remaining in the Soxhlet flask is washed by several portions of benzene to total 100 ml., which is finally collected in the separatory funnel.

Adsorption Columns.—For the first chromatographic column an adsorption tube 220 mm. in length and 20 mm. in inner diameter with a ground-glass joint and sealed-in perforated disk is employed. A 13-mm. layer of Hyflo-Supercel is placed in the bottom of the tube, upon which 20.0 Gm. of the alumina adsorbent are packed. Above the evenly distributed 90-mm. column (± 5 mm.) a thin layer (0.25 Gm.) of Hyflo-Supercel is added to prevent the column from cracking; and then the column is washed with 100 ml. of benzene.

A second chromatographic column makes use of the same type of tube. Two filter paper disks are placed on the top of the perforated glass, upon which 15.0 Gm. of silicic acid mixed with 50 ml. of chloroform are poured along a glass rod, while holding the filter paper in place. The column is left to settle for a few minutes and then stirred thoroughly in order to remove air bubbles and particles that are not gel-like, and also insure an even surface at the top of the column. After ten to fifteen minutes slight suction is applied and the gel-like adsorbent settles, making a column 110 mm. (± 5 mm.) \times 20 mm. Suction is controlled so as to avoid drying of the silicic acid. This can be detected as a white powder in the gel-like column.

A carefully prepared column retains the desired consistency throughout the entire process of elution. Dry particles decrease the rate of flow; and if the entire column dries out, elution becomes impossible.

The combined alcohol-benzene solution of colchicine present in the separatory funnel is passed through the first column. A chromatogram is developed which consists of three poorly separated bands. The top brown band is about 3 mm. wide, the middle reddish-brown band is about 4 mm. wide,

and the lowest band which contains colchicine is about 3 mm. wide and yellow in color.

The elution of colchicine is carried out in three stages with: (a) 100 ml. of chloroform; (b) 200 ml. of a 4% solution of methyl alcohol in chloroform; and (c) 200 ml. of a 10% solution of methyl alcohol in chloroform. After complete elution of the yellow band, 20-drop samples of the filtrate contain no colchicine.

The filtrate obtained from the separation is concentrated by distillation to about 1 ml. and transformed quantitatively in chloroform to a 100-ml. beaker, then evaporated to dryness on a water bath. The process of evaporation is repeated twice with 5-ml. portions of chloroform each time to remove all alcohol and benzene.

The washed and dried residue obtained from the filtrate of the first separation is dissolved in 10 ml. of chloroform and poured into the separatory funnel mounted above the second column. The beaker which contained this residue is washed with small portions of chloroform totaling 50 ml. and the combined solutions are passed through the silicic acid column. A chromatogram is developed consisting of the following bands (from top to bottom): 1. yellow, 2. red, 3. colorless, 4. red, 5. colorless, and 6. yellow-green-yellow.

Bands 2, 4, and 6, which contain impurities, are eluted with 100 ml. of chloroform, then with solutions of methyl alcohol in chloroform—first 125 ml. of 1% and finally 50 ml. of 2%. The colchicine present in band 1 remains at the top of the column and moves only slightly. During this process of elution, eluents consisting of more than 2% of methyl alcohol in chloroform should be avoided because higher concentrations of alcohol disturb the order of bands.

Colchicine is eluted as the final component, making use of 150 ml. of a 10% solution of methyl alcohol in chloroform. It is possible to increase the rate of elution with concentrations as great as 20%. Again, samples are taken to test completion of elution.

The eluate containing the colchicine is evaporated to dryness on a water bath. Ten ml. of methyl alcohol are added and evaporated in two successive steps to remove the chloroform.

Development of the Standard Curves.—The adsorption curves (Fig. 1) and the colchicine standard curve (Fig. 2) were derived by the following procedure: Samples of 0.5 mg., 1.0 mg., 2.5 mg., 3.0 mg., and 4.5 mg. of colchicine U. S. P. XIV were placed in 10-ml. volumetric flasks and dissolved in 0.1 ml. of concentrated nitric acid. A dark purple-blue-purple (PB-P) color (14) developed which changed to red-purple-red (RP-R), then finally red-yellow (RY), by the end of thirty minutes. This acid solution was washed with water containing 2.5 ml. of sodium hydroxide T. S., thereby developing a red color.

The flask was filled to volume with distilled water. The absorbances of these solutions were measured on the Beckman DU spectrophotometer, and these were used in plotting the absorption curves (Fig. 1). Single peaks of absorbance occurred at 350 millimicrons. The color densities for each concentration were then plotted and used to prepare the standard curve (Fig. 2).

Determination of Colchicine from Plant Tissue.—The residue from the chromatographic separations is transferred to a 100-ml. volumetric flask by means

of methyl alcohol and made up to volume. Ten-ml. samples of this solution are measured into weighing bottles, evaporated carefully to dryness on a water bath, and dried in an oven for sixteen hours at 105°. One-tenth ml. of concentrated nitric acid is added to the residue and allowed to stand for thirty minutes in order to develop the color. This acid solution is washed into a 50-ml. volumetric flask containing 2.5 ml. of sodium hydroxide T. S. The flask is filled to volume with distilled water, mixed thoroughly, and allowed to stand for fifteen minutes.

In order to have a solution within the proper concentration range, 3-ml., 5-ml., and 8-ml. aliquots are transferred to 10-ml. volumetric flasks and filled to the mark with water. The optical density of these solutions is read on the Beckman DU spectrophotometer at 350 m μ and the amount of colchicine is determined from the standard curve.

RESULTS

The sample of extracted colchicum seed mentioned above was divided into aliquots which were determined by both spectrophotometric and gravimetric methods. The results of these determinations are shown in Table I. The method was further compared with the official N. F. IX method and results are shown in Table II.

TABLE I.—RESULTS OF THE SPECTROPHOTOMETRIC AND GRAVIMETRIC METHODS FOR DETERMINING COLCHICINE IN COLCHICUM SEEDS

Sample number	Spectrophotometric method, mg./Gm. of seed	Gravimetric method, mg./Gm. of seed
1	3.0	3.3
2	3.0	3.6
3	3.1	3.4
4	3.0	4.3
5	3.1	3.7
6	3.0	3.8
7	3.6	3.7
8	3.6	3.6
9	3.2	3.2
10	3.1	3.3
11	3.0	3.3
12	3.0	3.1
Mean	3.1	3.5

TABLE II.—YIELD OF COLCHICINE OBTAINED BY THE N. F. IX METHOD AND BY THE EXPERIMENTAL METHODS (SPECTROPHOTOMETRIC AND GRAVIMETRIC)

Methods	Number of Determinations	Colchicine from 10 Gm. Colchicum Seed in mg.	Standard Deviation
N. F. IX	2	29.3	1.30
Experimental Spectrophotometric	12	31.6	2.40
Gravimetric	12	35.2	3.35

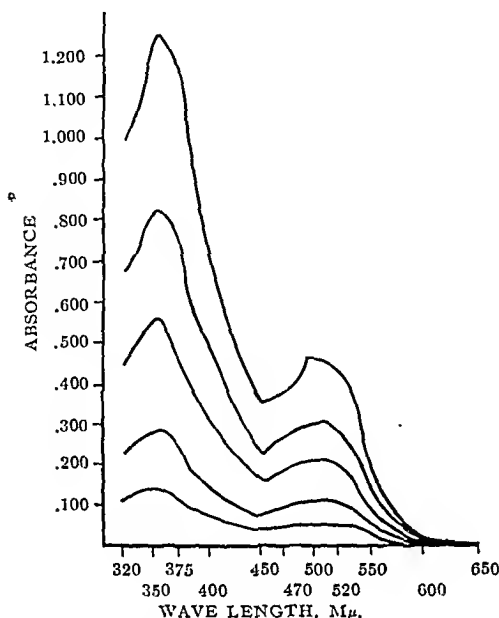


Fig. 1.—Absorption curves for colchicine following reaction with HNO₃, H₂O, and NaOH T.S. Amounts of colchicine present (reading from bottom to top of graph): I. 0.05 mg. in 10 ml. II. 0.1 mg. in 10 ml. III. 0.2 mg. in 10 ml. IV. 0.3 mg. in 10 ml. V. 0.45 mg. in 10 ml.

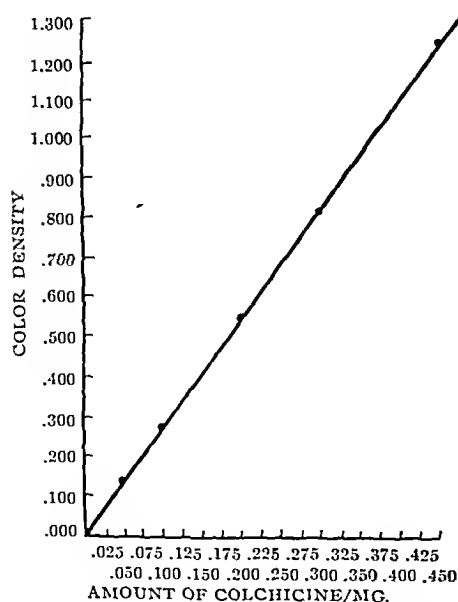


Fig. 2.—Standard curve obtained by plotting the spectrophotometric color density at 350 m μ against amount of colchicine in mg.

Sensitivity of method.—In order to test the degree of sensitivity of the method, a number of samples of colchicine were weighed, dissolved in the solvents mentioned above, and determined spectrophotometrically. The results are listed in Table III.

Determinations of Colchicine (U. S. P. XIV) were made by spectrophotometric and gravimetric methods, results of which are listed in Table IV.

TABLE III—SPECTROPHOTOMETRIC DETERMINATION OF UNKNOWN QUANTITIES OF COLCHICINE^a

Sample Number	Colchicine Taken, mg	Colchicine Determined, mg	Per Cent
1	5.2	5.0	96.1
2	2.1	2.0	95.7
3	2.5	2.4	96.0
4	2.5	2.5	100.0
5	1.0	1.1	110.0
6	1.0	1.0	100.0
7	1.4	1.4	100.0
8	0.2	0.2	100.0
9	4.5	4.4	97.8
10	1.1	1.2	109.0

^a Obtained from Mallinckrodt Chemical Works

TABLE IV—DETERMINATION OF COLCHICINE, U. S. P. XIV^a

Colchicine Taken, mg	Colchicine Recovered—Spectrophotometric, mg	Gravimetric, mg	Gravimetric Determination of Impurities Recovered from Chromatographic Columns, mg
50.9	48.3	52.5	3.9
51.4	47.9	51.5	4.0
Mean			
51.1	48.1	52.0	3.9
Standard deviation	0.28	1.87	..

^a Obtained from Mallinckrodt Chemical Works

DISCUSSION

The use of Struve's color reaction in detecting colchicine for the completion of extraction and elution gives excellent results. Since the color reaction consists of three phases, it is possible to eliminate similarly reacting compounds. This method is applicable only to solutions containing colchicine in high concentration, or their residues when evaporated to dryness.

After removal of fixed oil and fatty substances with petroleum benzene, the colchicine was extracted with ethyl acetate containing 1 ml. of ethyl alcohol. The ethyl acetate was chosen because it extracts a smaller amount of resinous substances

than does alcohol. The 1 ml. of ethyl alcohol was added to the ethyl acetate to prevent its hydrolysis. These two steps of extraction were used in order that the separation and purification, carried out solely on chromatographic columns, could produce a crude extract containing fewer interfering impurities during elution.

The crude extract contains a certain amount of resinous substances which are insoluble in chloroform and benzene, but soluble in methyl alcohol. However, some of the extract is also insoluble in the alcohol; consequently, the order of transferring the solution from the extraction flask to the column was by means of methyl alcohol followed by benzene. Alumina, used by Ashley and Harris (12) retained these, but it could not be used for the total purification, as it would not hold most of the non-resinous impurities. A second column, in which the adsorbent was silicic acid, removed these impurities.

The two adsorbents in different columns gave improved results. Alumina retained the resinous substances and some impurities; the colchicine being in the lowest band was eluted first. This eluate obtained from the first separation was easily processed and passed through the silicic acid column.

The Hyflo-Supercel used at the top and bottom of the alumina column serves only as a filter, since it does not adsorb colchicine.

An advantage of this method is that it does not require continuous care, and the many manual manipulations of other methods: e. g., the official N. F. IX method involves shaking with chloroform, causing emulsification of liquid if used too vigorously, too long, or too often; and repeated filtration is required to obtain clear solutions.

Differences obtained in the results from the gravimetric and spectrophotometric methods (Table I) indicate that there are colorless impurities remaining in the solutions, but they do not interfere with the spectrophotometric determination. The spectrophotometric results were always lower (Table IV) than the ones obtained by the gravimetric method, indicating that the impurities in solution did not absorb the transmitted light. A statistical comparison of the determinations presented in Table II indicates that their means are significantly different ($p = >.005$) and greater stress should be placed on results obtained by the spectrophotometric method.

REFERENCES

- (1) Coll, P. R., and Preiolini, P. J., *Rev. Farm. Buenos Aires*, **72**, 187 (1930).
- (2) "The National Formulary," 9th ed., AMERICAN PHARMACEUTICAL ASSOCIATION, Washington, D. C., 1950, pp. 164-165.
- (3) Santavy, F., *J. Pharm. and Pharmacol.*, **1**, 334 (1919).
- (4) Chemnitz, F., *J. Prakt. Chem.*, **118**, 29 (1928).
- (5) Cook, J. W., Johnston, T. Y., and Loudon, I. P., *J. Chem. Soc.*, **1**, 541 (1950).
- (6) Davies, E. C., "Year Book of Pharmacy," Vol. 10, AMERICAN PHARMACEUTICAL ASSOCIATION, Washington, D. C., 1921, pp. 393-395.
- (7) Self, P. A. W., and Cornfield, C. E., *Quart. J. Pharm. Pharmacol.*, **5**, 347-356 (1932).
- (8) Walaszek, E. J., Kelsey, F. E., and Gehrig, E. M. K., *Science*, **116**, 235 (1952).
- (9) Davies, E. C., and Grier, J., "Year Book of Pharmacy," Vol. 11, AMERICAN PHARMACEUTICAL ASSOCIATION, Washington, D. C., 1922, pp. 430-442.
- (10) Boyland, E., and Huntsman, M. E., *Biochem. J.*, **32**, 1204 (1938).
- (11) King, S. J., *This Journal*, **40**, 124 (1951).
- (12) Ashley, J. N., and Harris, J. D., *J. Chem. Soc.*, **2**, 677 (1914).
- (13) Struve, H., *Zeitschr. anal. Chem.*, **12**, 166 (1873).
- (14) "Munsell Book of Color," Munsell Color Co., Baltimore, Md., 1929.

A Study of Natural Sponge as a Disintegrating Agent in Compressed Tablets*

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Natural sponge (*Hippiospongia lachne*, de Laubenfels) has been studied as a disintegrating agent in compressed tablets. A brief description of the procedures used for cleansing, bleaching, and powdering the sponge prior to its incorporation in tablet formulas has been given. The disintegrating ability of powdered sponge in the formulas studied was found to be superior to that of cornstarch for tablets containing lactose, calcium gluconate, sulfadiazine, aluminum hydroxide, bismuth subnitrate, and sodium bicarbonate. Combinations of powdered sponge added during the granulation process and dried cornstarch added just before compression were more efficient for disintegration than powdered sponge alone.

IN A PREVIOUS PAPER (1), Gross and Becker showed that powdered sponge compared favorably with other disintegrating agents commonly employed in tablets. Since their work was limited to lactose tablets and a single percentage of sponge, it was the purpose of the present investigation to make a more detailed study of this agent.

For this investigation sheep's wool sponge (*Hippiospongia lachne*, de Laubenfels) was used because of its availability and commercial importance.

EXPERIMENTAL

The sponge used in this study was in the form of clippings which were trimmed from the sponges in the process of preparing them for market. These clippings contained large proportions of sand and shell as well as other foreign material. Because of the large amount of foreign material and the dark yellowish-brown color of the sponge, it was obvious that a thorough cleansing and decolorization was necessary before it could be used in a tablet formula. Preparation of the sponge for use as a disintegrating agent was accomplished in three steps, cleansing, bleaching, and powdering.

Cleansing of Sponge.—The sand and most of the other foreign material were easily removed from the sponge by washing and hand picking. Following this initial cleansing treatment, the sponge was found to still contain minute pieces of shell embedded within the fibrous network. These calcareous particles were removed by a second wash in a 2% solution of hydrochloric acid.

Bleaching of Sponge.—To decolorize the sponge, the cleansed material was first treated with a 1% solution of potassium permanganate. The sponge was next washed in an acidified solution of sodium bisulfite until the color had been sufficiently bleached. After rinsing in several changes of fresh

water, the sponge was dried and prepared for grinding.

Powdering of Sponge.—The sponge was ground in a Wiley mill using two screens, 2 mm. and 1 mm., respectively. The second milling was collected and stored for use in tablet formulas.

Absorption Study.—The mechanism through which powdered sponge and cornstarch accomplish their disintegrating action is most likely the same. Both agents swell when in contact with water and as a result are capable of rupturing tablets in which they are incorporated. Because absorption is a chief factor in this action, tests were conducted on powdered sponge and cornstarch to determine the comparative rate at which this absorption takes place. The moisture conditions were controlled through the use of constant humidity chambers. The materials were first dried to constant weight at 110° and then placed in each humidity chamber for a twenty-four hour period. The weight gain was calculated by reweighing the sample following this twenty-four hour interval and was recorded as per cent of sample weight. Results of these tests are reported in Table I.

TABLE I.—MOISTURE ABSORBED BY POWDERED SPONGE AND CORNSTARCH AT VARIOUS RELATIVE HUMIDITIES

Relative Humidity at 27°, %	Water Absorbed After 24 Hours Powdered Sponge, %	Cornstarch, %
30	9.22	8.13
45	12.21	10.33
60	15.43	12.69
70	17.65	14.14
80	21.36	16.12
95	29.51	20.61

Preparation of Tablets.—The tablet formulas used in this study are listed in Table II. Tablet batches were based on lots of 2,000 tablets for each formula. The medicinal ingredients selected as the active components of the tablets were represented by drugs of variable solubilities. As a control, lactose replaced all of the active medicinal agent in the tablet.

Four series of tablet formulas were prepared. The first, signified by the letter A in the batch number, contained 5% powdered sponge added during the process of granulation. The second series,

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signified by the letter *B* in the batch number, contained 10% cornstarch which was also added during the granulation process. A third series was made up of the same granulations as series *A* with an additional 2% dried cornstarch added to the dry granules just prior to compression, these formulas are signified by the letter *C* in the batch number. The amount of cornstarch added to this series was intentionally limited since the purpose of these formulations was not to determine the efficacy of cornstarch as a disintegrant, but rather to determine whether or not it would have any effect on the disintegrating ability of the powdered sponge which had been incorporated during the wet granulation. The formulas of the final series, signified by the letter *D* in the batch number, contained the same granulations as series *B* with an additional 2% dried cornstarch added to the dry granulation. Tablets in this last series were prepared and tested in order to obtain a fair evaluation of the data on a comparative basis.

Syrup U S P was used as the binder in the preparation of all granulations. This agent resulted in production of very fine granules. For lubrication, 2% magnesium stearate based on the weight of the finished tablet was used in all formulations. A Stokes rotary, Model B-2, tablet machine was used to compress the completed granulations. The tablets were prepared with standard concave $\frac{3}{8}$ -inch punches and dies.

To prepare the granulations, the powdered materials were placed in a Stokes mixer and allowed to mix for one hour. The resultant mixture was sieved through a No. 40 mesh screen.

In order that the percentage of active ingredient and disintegrator remained constant for each series of formulas, it was necessary to predetermine the proportion of binding agent needed for each granu-

lation. This was accomplished through a preliminary experiment on a small amount of each formula. With the proportion of granulating agent determined for the actual granulation process, the adjustment of diluent to obtain a specific tablet weight was calculated.

The thoroughly mixed powders were put in a Readco Dough Type Mixer and the granulating solution was slowly added with constant mixing. The moist mass was granulated by passing it through a No. 8 mesh screen. The coarse granules were spread in thin layers on paper-covered trays and allowed to dry in a circulating hot-air oven at 60°. The time required for drying varied with the nature of the granulation, however, as a rule the time ranged between eight to ten hours. Following the drying period, the granulation was again screened, this time through a No. 14 mesh sieve.

For those granulations that had a tendency to produce excessive "fines" when completely dried, it was necessary to screen the coarse granulation when it was about $\frac{3}{4}$ or $\frac{2}{3}$ dry. After screening a partially dried granulation, it was placed back into the oven and allowed to completely dry with a minimum of fines.

Lubrication of the granules was carried out immediately prior to compression. The lubricant was forced through a No. 80 mesh screen before being added to the granulation.

Measurements of Hardness and Disintegration Time.—To measure the comparative hardness of the tablets prepared in this investigation, the Monsanto Hardness Tester was used. Reported values are averages of five determinations and are stated to the nearest 0.5 Kg. Since tablet hardness has an influence on the rate of disintegration, an attempt was made to maintain the tablet hardness

TABLE II—TABLET FORMULAS^{a, b}

Batch No	Active Ingredient	%	Binder Syrup	Disintegrant	Sponge,	Starch,	Tablet Weight ^c mg
			U S P, %				
I-A	78	8 Lactose	14	2	5		375
I-B	72	7 Lactose	15	3		10	375
I-C	78	8 Lactose	14	2	5	2	383
I-D	72	7 Lactose	15	3		12	420
II-A	50	0 Bismuth Subnitrate	16	5	5		375
II-B	50	0 Bismuth Subnitrate	17	4		10	375
II-C	50	0 Bismuth Subnitrate	16	5	5	2	383
II-D	50	0 Bismuth Subnitrate	17	4		12	420
III-A	50	0 Sulfadiazine	18	0	5		350
III-B	50	0 Sulfadiazine	21	3		10	350
III-C	50	0 Sulfadiazine	18	0	5	2	357
III-D	50	0 Sulfadiazine	21	3		12	392
IV-A	50	0 Aluminum Hydroxide	24	3	5		400
IV-B	50	0 Aluminum Hydroxide	25	0		10	400
IV-C	50	0 Aluminum Hydroxide	24	3	5	2	408
IV-D	50	0 Aluminum Hydroxide	25	0		12	448
V-A	30	0 Calcium Gluconate	13	6	5		400
V-B	30	0 Calcium Gluconate	13	5		10	400
V-C	30	0 Calcium Gluconate	13	6	5	2	408
V-D	30	0 Calcium Gluconate	13	5		12	448
VI-A	45	0 Sodium Bicarbonate	12	1	5		350
VI-B	45	0 Sodium Bicarbonate	13	7		10	350
VI-C	45	0 Sodium Bicarbonate	12	1	5	2	357
VI-D	45	0 Sodium Bicarbonate	13	7		12	392

^a All percentages of series *A* and *B* were based on the weight of the finished tablet. In series *C* and *D* an additional 2% dried cornstarch was added to the tablet weight of those formulas in series *A* and *B*, respectively.

^b The remaining weight of the tablet was furnished by the diluent and lubricant.

^c The weight variation of the tablets was within $\pm 5\%$ of the stated weight.

within a range of 6.0 to 7.5 Kg. Tablets in this hardness range were found to be resistant to breaking and chipping when subjected to friability tests simulating the most extreme handling conditions. In addition, it was felt that a hard tablet would better reveal the disintegration ability of the agents studied.

The disintegration tests were conducted in accordance with the U. S. P. directions (2). A tablet was considered as being disintegrated when it had completely passed through the screen of the basket-rack assembly. Three determinations were made on all tablets tested and the average time was calculated and recorded in minutes.

The average disintegration time as well as the average hardness of the various tablet batches are shown in Table III.

TABLE III.—AVERAGE HARDNESS AND DISINTEGRATION TIME OF TABLETS MEASURED IMMEDIATELY AFTER COMPRESSION

Batch No. ^a	Hardness, Kg.	Disintegration Time, Min.
I-A	6.0	3.6
I-B	6.5	21.0
I-C	7.5	0.9
I-D	7.0	19.7
II-A	7.0	23.2
II-B	6.0	73.9
II-C	7.5	4.0
II-D	7.5	71.2
III-A	6.5	6.3
III-B	6.5	98.5
III-C	7.0	1.5
III-D	7.0	94.4
IV-A	7.0	6.1
IV-B	6.5	88.5
IV-C	6.0	0.7
IV-D	6.5	84.3
V-A	6.5	13.8
V-B	6.0	21.1
V-C	7.5	2.3
V-D	7.0	58.7
VI-A	6.5	2.1
VI-B	7.0	20.4
VI-C	7.0	0.5
VI-D	7.0	19.0

^a Key to Numerals.—The numeral represents the medication used in the formulation; I—lactose, II—bismuth subnitrate, III—sulfadiazine, IV—aluminum hydroxide, V—calcium gluconate, VI—sodium bicarbonate.

Key to Letters.—The letter used in conjunction with the numeral indicates the disintegrant or combination of disintegrants used in the formula: A—5% powdered sponge added during the wet granulation; B—10% cornstarch added during the wet granulation; C—2% dried starch added to the dry granulations of series A; D—2% dried starch added to the dry granulations of series B.

DISCUSSION AND CONCLUSIONS

The effectiveness of powdered sponge as a disintegrating agent lies in its ability to absorb liquids. Studies conducted to determine the absorption characteristics of powdered sponge and cornstarch indicate, as shown in Table I, that the moisture absorbed by these agents increased with increased relative humidities. The increase in moisture content by each agent may be illustrated by an S-shaped curve; the curve being more pronounced for powdered sponge, indicating a greater capacity for absorption by this agent.

The mechanism through which sponge absorbs water and other liquids may be attributed to a com-

bination of adhesive and cohesive forces. First, due to adhesion, there is a wetting of the entire surface of the sponge creating an enormous liquid surface. The surface tension effect then tends to reduce this surface to a minimum value through the movement of the liquid into myriads of minute interstitial spaces and capillaries of the sponge skeleton. This causes the sponge particles to swell to a wet volume much greater than their dry volume resulting in an effective disintegrant.

A careful study of the data in Table III reveals two important facts pertaining to the efficacy of powdered sponge as a disintegrating agent for compressed tablets. First, it is noted that tablets in series A containing 5% powdered sponge as the disintegrant, disintegrated in all cases more quickly than those tablets in series B which possessed 10% cornstarch as the disintegrating agent. Secondly, and of particular interest, is the fact that when an additional 2% dried starch was added to the dry granulation of series A, the time required for disintegration of the tablets was markedly reduced. Although it may seem that the additional 2% dried cornstarch in tablets of series C was totally responsible for the increased rate of the disintegration, this is found not to be true when the disintegration times of the four series of tablets are compared. In making this comparison, it is noted that the reduction in disintegration time for tablets containing an additional 2% dried starch was more pronounced for tablets having powdered sponge as the incorporated disintegrant than for those having 10% cornstarch. This is especially true for those formulas in group II and V. The dried cornstarch, therefore, in addition to serving as a disintegrant *per se*, exhibited a potentiating effect on the disintegrating action of the powdered sponge. This may be explained by taking into consideration the structure of the finished tablets. In formulas of series A, the powdered sponge is intimately mixed throughout the tablet granules thereby necessitating intergranular absorption of the liquid media before swelling of the sponge particles takes place. However, when the 2% cornstarch was added to the dry granulation just prior to compression, since it was located around the outside of the granules rather than within the granules, it served to reduce the time required for the disintegration fluid to seep through the outer layers of the tablet granules. This allowed the sponge particles, especially in the granules not occupying the outermost layers of the tablet, to have a more ready access to the disintegration fluid, and, as a result, to decrease the time required for liquid absorption to take place. This in turn increased the rate of swelling of the sponge particles and likewise the disintegration of the tablet. When used in this manner, the starch itself, although acting to some extent as a contributing disintegrant, served principally as an activator to disintegration by hastening the action of the powdered sponge.

The results in Table III also indicate, as has been noted by several other investigators, that the medicament present has a decided influence on disintegration.

REFERENCES

- (1) Cross, H. G., and Becker, C. H., *THIS JOURNAL*, 41, 1957(1952).
- (2) "The United States Pharmacopeia," 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 937.

The Detection of 1-Ethynyl Cyclohexyl Carbamate in Toxicological Specimens and its Separation from a Mixture of Other Drugs

By GEORGE R. NAKAMURA

Three different solvent mixtures are presented for the separation of Valamin in combination with some common pharmaceutical compounds, principally, bromural and morphine. Rf's of Valamin and some drugs and their respective colors on the chromatogram are described for three different solvent mixtures. Color tests for Valamin with concentrated sulfuric acid and other reagents using sulfuric acid as a solvent are described.

THE COMMON USE of 1 ethynyl cyclohexyl carbamate, hereafter indicated in this paper as Valamin,¹ as a sedative is acknowledged today, and in a number of cases studied in this laboratory, the drug has been taken by individuals in an attempt to commit suicide. In a few cases gastric washings contained a mixture of Valamin and bromisovalum (isobromovalerylurea).

Valamin seems to be rapidly metabolized by the body as suggested by the work of Swanson, *et al* (1) on dogs. We have not been able to recover any Valamin in tissue, blood, and urine specimens obtained from autopsy cases. Bromisovalum was traced in the liver and in the kidney in cases where stomach washings contained large amounts of Valamin and bromisovalum.

Since Valamin shows extremely low ultraviolet and infrared absorption and no fluorescence activity the detection of the drug was carried out by means of color tests and chromatography.

The precipitation method with ammoniacal silver nitrate of Langecker, *et al* (2) proved useful but lacked specificity and sensitivity. Other confirmatory tests were sought. Various reagents commonly employed in the toxicology laboratory were tested and our preliminary search indicated that concentrated sulfuric acid, which produces a vivid orange red color with Valamin could serve as a fairly sensitive reagent. Comparatively few pharmaceutical compounds react with concentrated sulfuric acid to produce this characteristic color. Some reagents using H₂SO₄ as a solvent were tested with varied results.

EXPERIMENTAL

For the separation of Valamin from bromural and other drugs, paper chromatography was applied. It

* Received October 1 1957 from the Department of Chemistry 406th Medical General Laboratory APO 343 San Francisco Calif.

¹ This drug is available commercially in Japan as Valamin a product of Schering in Germany and the same drug manufactured by Eli Lilly and Co Indianapolis 6 Ind is called Valmid. Inasmuch as the use of Valamin is more common in Japan it was employed for this investigation.

was apparent from the start that to seek a "universal solvent mixture" to resolve at least a dozen of the drugs which are routinely analyzed in this laboratory would prove futile. An attempt was made to separate some of these compounds which would appear in the basic chloroform extracts, principally morphine, meperidine and bromisovalum.

Ammoniacal silver nitrate spray (3) was employed not only to develop spots and to determine their mobility in the solvent mixtures, but also to differentiate the various drugs by colors.

Extraction.—To 10 Gm amounts of tissue, gastric contents, or blood, 10 ml of water was added, and the mixture was homogenized in a blender. The pH of the resulting homogenate was adjusted to 4-5 and the homogenate was heated in a water bath at 100° for thirty minutes. After centrifugation, the supernatant was collected, and the pH was adjusted to 8 with ammonium hydroxide. It was then shaken twice with 50 ml of chloroform and the aqueous layer was discarded. The chloroform extract was collected and washed with phosphate buffer, pH 7.6. The chloroform layer was filtered through filter paper containing Celite,² then evaporated gently to dryness in the steam bath.

Ten milliliters of a urine sample was extracted with chloroform at pH 8.0 without prior steam heating. The chloroform extract was treated as above with Celite and filtered. The filtrate was evaporated to dryness.

Chromatography.—A cylindrical tank 46 cm in height and 22 cm in diameter fitted with a ground glass lid was employed for ascending chromatography. This tank accommodates a 44 x 48 cm sheet of Whatman No. 1 filter paper, rolled and stapled in such a manner that the edges do not contact each other.

The paper was spotted with extract residue containing not less than 50 µg of Valamin on a base line 3 cm from the bottom of the paper. The standards listed in Table I were dissolved in ethanol and applied as 50 microgram spots on the same base line. The ascending chromatogram was allowed to develop for eighteen hours at room temperature. The paper was dried, then mist sprayed with 0.5% aqueous solution of sodium carbonate and dried again. Then the sheet was sprayed with 2% silver nitrate in 10% ammonia water and was dried in direct sunlight to produce a clear color development. A photo-flood bulb, about 250 watts, could be used with the same effectiveness.

² Johns Manville

To acidify the atmosphere of the chamber, an evaporating dish containing glacial acetic acid was placed on the bottom of the tank. The paper was sprayed with acetic acid immediately prior to introduction into the tank.

Color Test.—A small amount of residue was placed on a white porcelain plate and a drop of color reagent was added. The color development was observed for a one minute period. Any gradual change of color was noted. Then the plate was warmed on a hot plate and any further change in the color was observed.

RESULTS

The results of chromatographic separation of Valamin and eight other drugs in three different solvent mixtures are presented in Table I. Color test results with concentrated sulfuric acid and reagents (4) using sulfuric acid as a solvent are shown in Table II.

chloroform extracts of urine and blood specimens or in autopsy tissues.

It is assumed, at the present, that Valamin is metabolized very rapidly in man through the destruction of the ethinyl group. Experiments with rat tissue slices and on dog blood levels bear out the metabolic lability of this structural group (5).

While very few common pharmaceutical compounds yield a red color in reaction with sulfuric acid, drugs such as chlorpromazine and dimenhydrinate which may occur in the basic chloroform extract should be eliminated as possibilities. Many of the organic bases such as morphine, codeine, heroin, meperidine, ephedrine, amphetamines, methyl parafynol, prisolone, brucine, and chloroquin do not form a red complex with sulfuric acid.

Generally barbiturates and ureides do not react with the acid to form colors but hexobarbital and cyclobarbital do produce an orange-brown to reddish-brown color.

For the chromatography procedure, many labo-

TABLE I.—TYPICAL R_f VALUES OF VALAMIN AND SOME COMMON DRUGS USING THREE DIFFERENT SOLVENT MIXTURES

Drug	Color	Isoamyl Alcohol Paper Impreg- nated with Glacial Acetic Acid	Cyclohexane Paper Impreg- nated with Glacial Acetic Acid	Isoamyl Alcohol (Saturated with Conc. NH_4OH), Conc. Ammonium Hydroxide, 3:1 v/v
Valamin	Yellow	0.69	0.65	0.22
Bromisovalum	Pink-Red	0.65	0.69	0.78
Morphine	Blue-Black	0.42	0.22	0.80
Dimenhydrinate	Violet	0.58	0.29	0.25
Benadryl	Violet	0.58	0	0.21
Meperidine	Purple	0.49	0.19	0.24
Codeine	Brown	0.43	0.21	0.75
Chloroquin	Blue-Green	0.15	0.27	0.24
Chlorpromazine	Violet	0.59	0.19	0.65

TABLE II.—COLOR REACTIONS OF VALAMIN IN CONCENTRATED SULFURIC ACID AND SOME REAGENTS (4) USING SULFURIC ACID AS A SOLVENT

Reagent	Color	After Heating
H_2SO_4 , conc.	Orange	Orange to Red
Mecke	Yellow	Brown
Marquis	Brown	Brown
Froede	Cherry-Red to Blue	Blue to Green
Rosenthaler	Red	Brown
Erdman	Colorless	Colorless
Mandelin (Vanillin- H_2SO_4)	Brick-Red to dull-Orange	Brown
Dimethyl amino- benzaldehyde	Orange-Red	Blood-Red
Molisch	Brick-Red	Brown
Phenol-sulfuric acid	Yellow	Orange

DISCUSSION

In a number of cases in which several hundred-milligram quantities of Valamin were detected in the gastric contents, no substances which gave positive reactions with sulfuric acid were located in the

ratory alcohols were tested in various proportions with water in both acidic and basic media to obtain the best possible separation of Valamin from bromisovalum and morphine. Alcohols which were empirically tested in various mixtures were methanol, ethanol, *n*-propanol, iso-propanol, *n*-butanol, isobutanol, *n*-amyl alcohol, and iso-amyl alcohol. Other solvent mixtures contained acetone, chloroform, ethyl acetate, pyridine, propylene glycol, cyclohexane, benzene, and collidine. Schmall, *et al.* (6), introduced an elegant paper chromatography method by which organic bases were separated on a multibuffered paper. This laboratory has found this technique useful for the separation of morphine and codeine, but Valamin could not be immobilized in any of the buffer zones above pH 2.0.

It was discovered that impregnating the paper with glacial acetic acid prior to irrigation provided clear, compact spots. Merely saturating a paper in an atmosphere of acetic acid, even for a twelve-hour period, was not comparable to the effect produced by actually wetting the paper with the acid.

A mixture of Valamin and bromisovalum proved to be very difficult to separate as the two compounds seem to possess identical mobility patterns in nearly all of the solvent preparations tried except in a two-phase system of isoamyl alcohol and ammonia. Three solvent mixtures which gave the best separa-

tion of Valamin with respect to eight other drugs are presented in Table I. The R_f values presented in the table do not imply that the various substances, including Valamin, listed herein can be identified by these values alone, inasmuch as the R_f differences are so small between certain compounds

Fortunately bromisovalum and Valamin each reacted to ammoniacal silver nitrate with distinct colors (see Table I). Identification of drugs by the color of their spots on a chromatogram proved to be another useful criterion.

The color development of Valamin with concentrated sulfuric acid is extremely sensitive but not specific, however the results obtained from the chromatography procedure were of aid in the elimination

of the possible presence of the few compounds which produce colors similar to Valamin in sulfuric acid.

REFERENCES

- (1) Swanson, E. E., Anderson, R. C., and Gibson, W. R., *THIS JOURNAL*, 45, 40 (1956).
- (2) Langecker, H., Schumann, H. J., and Junkman, K., *Arch. Exptl. Pathol. Pharmacol.*, 219, 130 (1953).
- (3) Ueda, K., *Sci. and Crime Detection (Japan)*, 8, 26 (1955).
- (4) Gonzales, T. A., Vance, M., Helpert, M., and Umberger, G. J., "Legal Medicine Pathology and Toxicology," Appleton-Century-Crofts, Inc., New York, N. Y., 1954, p. 1191-1255.
- (5) Perlman, P. L., and Johnson, C., *THIS JOURNAL*, 41, 13 (1952).
- (6) Schmall, M., Wollisch, E. G., and Shafer, E. G. G., *Anal. Chem.*, 29, 1373 (1956).

A Comparison of the Pharmacological Properties of Two Nitrated Cyclohexanols*

By WILLIAM J. FLEMING,[†] EDWARD J. ROWE, and DONALD B. MEYERS

The nitrate esters of two isomeric cyclohexanols, *meso*-inositol and scyllitol, were prepared and their pharmacological effects studied. The compounds exhibited the typical organic polynitrate action of depressing smooth muscle when tested on various isolated tissues such as the rabbit intestine, guinea pig ileum, and rat and rabbit lungs. A typical vasodepressor action was obtained from anesthetized cats and rabbits. The acute toxicity of the nitrates was also determined. The results of this study showed the *meso*-inositol nitrate to be the more active of the two compounds in depressing smooth muscle of the organs studied. No species variation was noted.

SINCE THE INTRODUCTION of glyceryl trinitrate as a medicament nearly one hundred years ago, various organic polynitrates have been utilized as smooth muscle relaxants; however, relatively little is known regarding their mechanism of action. Since the compounds exhibit activity similar to the metallic and organic nitrites, it was postulated by Hay (1) and substantiated by others (2, 3) that the organic nitrates were converted to nitrite ion in the blood and exerted their physiological actions by this mechanism.

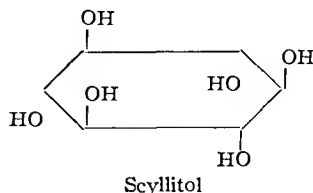
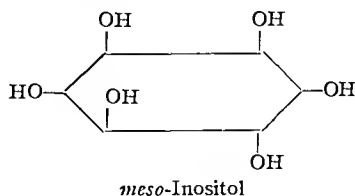
More recently, Krantz and co-workers, in a series of papers (4-7), offer evidence that the organic polynitrates act as intact molecules and not through hydrolysis and reduction to nitrite. This assumption is also held by Marshall (8).

Thus far, little has been done in correlating the spatial configuration necessary to obtain maximum depressor activity of the organic nitrates. It is known that methyl nitrate has little depressor properties while most of the active members of the series are derived from polyalcohols. However, Marshall found that the

arrangement of the nitrate groups on a series of isomeric hexahydric alcohols did not appear to influence the activity of the compounds (8). In this study, a comparison of the relative potency of two isomeric nitrated cyclohexanols was undertaken.

EXPERIMENTAL

Preparation of the Nitrated Products of *meso*-Inositol and Scyllitol.—The spatial configurations of the two isomers of inositol which were used in this study are given below (9).



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Inositol Nitrate.—*meso*-Inositol nitrate was prepared by dissolving 5 Gm. (0.028 moles) of anhydrous *meso*-inositol in 10 ml. (0.22 moles) of cold, fuming nitric acid. After complete solution was obtained, 10 ml. of fuming sulfuric acid was slowly added with constant stirring. During this time a white, magma-like mass formed. The temperature maintained throughout the synthesis was 0–5°. The precipitate was diluted with cold distilled water, filtered, washed thoroughly with cold water, and dried under a carbon dioxide atmosphere.

The product, when recrystallized two times from a 2:1 v/v mixture of 95% ethanol and water and two times from benzene, melted at 119–121°¹ which is in agreement with that recorded for the hexanitate. A yield of 31.6% was obtained.

meso-Inositol nitrate is a fine, white, crystalline powder which decomposes readily in the dry form when exposed to the atmosphere at room temperature. It is insoluble in water but is soluble in alcohol, propylene glycol, acetic anhydride, and warm benzene.

Anal.—Calcd. for $C_6H_8N_6O_{18}$: N, 18.67. Found: N, 17.48 (Kjeldahl).

Scyllitol Nitrate.—The nitrated product of scyllitol was prepared by adding, with stirring, 4 Gm. (0.022 moles) of scyllitol to 10 ml. (0.22 moles) of fuming nitric acid. Upon standing, a white, magma-like mass formed which did not dissolve in the acid. Fuming sulfuric acid, 10 ml., was slowly added with constant stirring. The temperature was maintained at 0–5° throughout the procedure. The precipitate was diluted with ice water, filtered, washed thoroughly with cold water, and dried under a carbon dioxide atmosphere.

The final product is a dense, white powder which melted at 123–125° with decomposition. A yield of 90.75% was obtained. It is insoluble in water and the common organic solvents.

Anal.—Calcd. for $C_6H_8N_6O_{18}$: C, 16.01; H, 1.33; N, 18.66. Found: C, 18.05; H, 1.57; N, 15.84 (Kjeldahl).

PHARMACOLOGY

Since the organic polynitrates exhibit relaxing actions on many organs containing smooth muscle, a pharmacological comparison of the two compounds was made by studying their relative effects on the vascular system, small intestine, uterine muscle, tracheal chain, and isolated lung.

In all studies except that of acute toxicity, the compounds were administered in a propylene glycol vehicle. *meso*-Inositol nitrate was soluble in a concentration of 10 mg./ml., while the scyllitol nitrate formed a uniform suspension. Acute toxicity studies of both drugs were carried out with the compounds suspended in a 0.5% methylcellulose solution.

PERFUSION STUDIES

Isolated Rabbit Intestine.—Randomly selected segments of small intestine from non-fasting, albino rabbits were isolated and suspended in water baths containing aerated Tyrode's solution at a temperature of 37–38°. The volume of the bath was 100 ml.

throughout the study. The free ends of the segments were attached to lever-arms for kymograph recording.

The results of this experiment showed that *meso*-inositol nitrate, in doses of 0.5–1.0 mg., was active in lowering both the tone and amplitude of intestinal muscle while comparable concentrations of scyllitol nitrate exhibited little activity.

The vehicle, propylene glycol, caused an immediate slight fall in tone which was followed by an increase in tone and a decrease in amplitude when administered in doses of 3 ml.

Antagonism Against Histamine Induced Spasm of Isolated Guinea Pig Ileum.—Ileal segments of nonfasting guinea pigs were isolated and suspended in a water bath containing aerated Locke-Ringer's solution at a temperature of 37–38°. The bath volume was 100 ml. throughout the experiment. The system was organized for kymograph recording in the usual manner.

After tracings of normal ileal movements were obtained, 0.1 ml. of histamine acid phosphate, 1:1000, was added to the preparation to produce an immediate ileal spasm. The drug was left in contact with the tissue for two and one-half minutes. The segment was then washed and the contractions allowed to return to normal. The same procedure was followed in subsequent trials, with the alteration that thirty seconds after the dose of histamine acid phosphate, *meso*-inositol nitrate or scyllitol nitrate was added in varying concentrations. Their effects of alleviating the spastic ileum were noted over a time interval of two minutes.

It was determined by this study that *meso*-inositol nitrate was 1.46 times as active as scyllitol nitrate in doses of 1.5 mg., and 1.99 times as active in doses of 1.0 mg., in relaxing the spastic ileum after the addition of histamine.

Guinea Pig Tracheal Chain.—The tracheae of two albino, female guinea pigs were used to prepare a chain according to the method of Castillo and DeBeer (10). The chain was suspended in aerated Van Dyke-Hastings solution modified by the addition of 0.05% dextrose. The bath was maintained at a temperature of 37–38° and a volume of 100 ml. throughout the experiment.

Histamine acid phosphate, 0.5 mg., was added to the bath to cause a contraction of the chain. Neither of the compounds under study, in doses up to 3 mg., produced any antagonism to histamine induced tracheal spasm when administered before or after the histamine.

Isolated Guinea Pig Uterus.—Uterine segments of nonpregnant guinea pigs were obtained and suspended in aerated Locke-Ringer's solution at a temperature of 37–38°. The bath volume was 100 ml.

Doses as high as 3 mg. of either *meso*-inositol nitrate or scyllitol nitrate had no activity in relaxing the tissue or antagonizing contractions caused by posterior pituitary, U. S. P., in concentrations of 1×10^{-3} units/ml.

Isolated Lung Perfusion.—The lungs and trachea were removed from a rabbit or rat and placed on a T-type cannula. Modified Locke-Ringer's solution was then perfused through the lung at a positive, constant pressure and a temperature of 37–38°. After expressing the residual air in the lungs through the sidearm of the cannula, the drugs were injected into the perfusion fluid at a rate of 1 ml./min. and

¹ The melting points were determined with a Fisher-Johns apparatus.

their actions in altering the rate of outflow were noted.

It was found that *meso*-inositol nitrate in doses of 5 mg. and 10 mg. was active in increasing the lung outflow in the rat, but the results were not conclusive in the rabbit study. Scyllitol nitrate was somewhat active in increasing outflow in both the rat and rabbit; however, the compound was not as potent as its *meso*-inositol isomer in duration of action or outflow when compared in equal doses on the isolated rat lung.

BLOOD PRESSURE STUDIES

Anesthetized Rabbit.—Six albino rabbits of either sex and weighing 3.5–4.1 Kg. were anesthetized with urethane, 1.5 Gm./Kg., rectally and maintained throughout the experiment with intravenous injections of secobarbital sodium as needed. The animals were prepared for blood pressure and respiration recording by cannulating the right common carotid artery and trachea. All injections were introduced through the marginal ear vein.

Both of the drugs were effective in lowering blood pressure in doses of 5 mg. (Table I). The fall in blood pressure took place within ten seconds after the injection had begun and was accompanied by an increase in the rate and amplitude of respiration. Doses lower than 5 mg. produced rather transient depressions in pressure. The same general responses were obtained after double vagotomy.

Anesthetized Cat.—Three female cats weighing 2.1–2.9 Kg. were anesthetized with phenobarbital sodium, 175 mg./Kg., by intraperitoneal injection. The animals were prepared for blood pressure and respiratory studies in the usual manner. All injections were introduced through the left external jugular vein.

The results of this study are essentially the same as observed in the rabbit and are tabulated in Table I. It can be seen that *meso*-inositol nitrate is the more active of the two compounds from the standpoint of the amount of fall in pressure and duration of action.

TABLE I.—BLOOD PRESSURE STUDY IN THE CAT AND RABBIT

Drug	Dose, mg.	Av. ^a Fall in Systolic B.P., mm.		Av. ^a Duration of Action, min.	
		Rab-bit	Cat	Rab-bit	Cat
m-Inositol Nitrate	5	23	..	8.8	..
Scyllitol Nitrate	5	19	..	5.3	..
m-Inositol Nitrate	10	32	33	9.6	11
Scyllitol Nitrate	10	21	21	6.6	9
m-Inositol Nitrate	15	30	31	5.1	9.5
Scyllitol Nitrate	15	19	22	6.2	7.5

^a Av. in 6 rabbits and 3 cats.

Acute Toxicity-Intraperitoneal LD₅₀.—Albino mice, weighting 14–20 Gm., were injected intraperitoneally with the drugs suspended in a 0.5% methyl

cellulose solution. The test animals were placed in cages by dose and observed over a period of 72 hours.

The majority of mortalities occurred after 24 hours and before 48 hours. The only apparent toxic sign was a cyanosis seen in the tail four hours after injection.

The LD₅₀ and standard error of the compounds was calculated by the method of Bliss (11). The LD₅₀ of *meso*-inositol nitrate was found to be 0.957 ± 0.057 Gm./Kg. The LD₅₀ of scyllitol nitrate was computed to be 1.267 ± 0.088 Gm./Kg.

DISCUSSION

The data obtained from the pharmacological comparison of the nitrated products of *meso*-inositol and scyllitol indicates that *meso*-inositol nitrate is the more active of the two compounds in relaxing the smooth muscle of the various organs studied from the standpoint of the amount of activity and duration of action.

In comparing the relative potency of the two drugs, several factors should be considered: spatial relationships, solubility, and the degree of nitration obtained.

It can be seen from the structural configurations that *meso*-inositol has three adjoining hydroxyl groups on the same side of the plane of the cyclohexane ring, while the hydroxyl groups of scyllitol alternate above and below the plane of the ring; and therefore, none of the groups are directly adjacent. As yet, the relationships necessary for the organic polynitrates to exhibit maximum depressor activity is not known, but since methyl nitrate and ethylene glycol dinitrate have little smooth muscle relaxant properties while activity increases with higher members of the series, it may be possible to draw some correlation of activity with the number of adjacent nitrate groups on the molecule.

Secondly, from the previously described physical properties of the two compounds, it is evident that scyllitol nitrate is generally the more insoluble. This factor could well play a part in limiting the activity of the drug by altering its contact and fixing abilities with the smooth muscle tissue.

Although analytical determinations indicate that *meso*-inositol nitrate was more completely nitrated than the scyllitol derivative, the importance of this factor is not known; however, in most cases the pharmacological response produced by *meso*-inositol nitrate could not be obtained with even twice the quantity of scyllitol nitrate.

REFERENCES

- (1) Hay, M., *Trans. Roy. Soc. Edinburgh*, 32, 67(1883).
- (2) Leech, D. I., *Brit. Med. J.*, 1, 1305(1893).
- (3) Herrman, R. F., Leake, C. D., Loevenhart, A. S., and Muehlberger, C. W., *J. Pharmacol. Exptl. Therap.*, 27, 259(1926).
- (4) Krantz, J. C., Jr., Carr, C. J., Forman, S., and Ellis, F. W., *ibid.*, 67, 187(1939).
- (5) *Ibid.*, 67, 191(1939).
- (6) Krantz, J. C., Jr., Carr, C. J., Forman, S., and Cone, N., *ibid.*, 70, 323(1940).
- (7) Rath, M. M., and Krantz, J. C., Jr., *ibid.*, 76, 33(1942).
- (8) Marshall, C. R., *ibid.*, 83, 106(1945).
- (9) Pigman, W. W., and Woffrom, M. L., "Advances in Carbohydrate Chemistry," Vol. 3, Academic Press Inc., New York, 1948, p. 47.
- (10) Castillo, J. C., and DeBeer, E. J., *J. Pharmacol. Exptl. Therap.*, 90, 104(1947).
- (11) Bliss, C. I., *Quart. J. Pharm. and Pharmacol.*, 11, 192(1938).

Peristaltic-Stimulating and Fecal-Hydrating Properties of Dioctyl Sodium Sulfosuccinate, Danthron, and Cascara Extracts in the Mouse and Rat*

By PAUL M. LISH and KENDRICK W. DUNGAN

Utilizing the criteria of fecal-hydration and peristaltic-stimulation the potency and properties of Colace (dioctyl sodium sulfosuccinate), danthron N. F., and four cascara extracts were investigated in mice and rats. The results suggested that Peristim (Casanthranol), would be the most suitable peristaltic-stimulating agent as an adjunct to Colace therapy. Peristim was approximately 3 times as active as Cascara Sagrada Extract N. F. The peristaltic-stimulating action of Peristim was demonstrated in normal rats, in rats after postoperative intestinal stasis, and after ganglionic blockade. Peristaltic-stimulating properties could not be demonstrated for danthron in rats. Danthron and Peristim showed similar abilities to induce fecal-wetting in mice but this action of danthron, unlike that of Peristim, was strongly inhibited by ganglionic blockade. Colace was approximately 3.5 and 2.2 times as potent as Peristim in producing fecal-wetting in mice and rats respectively. The fecal-hydrating action of Colace was inhibited in the mouse and rat by chlorisondamine and atropine, but not by bile duct cannulation in the rat. As assessed by the mouse fecal-hydrating test, Peristim and Colace were additive while danthron and Colace were not.

THE ORAL ADMINISTRATION of dioctyl sodium sulfosuccinate, Colace¹ to humans has been shown to cause effective fecal softening (1, 2) with otherwise negligible pharmacological effects (3). It has been suggested that in some patients a laxative agent with peristaltic-stimulating action would be a desirable adjunct to Colace therapy (2, 4). The ideal peristaltic stimulant should be effective in spite of a variety of normal or abnormal conditions interfering with the neuronal or muscular function of the intestine. Cascara sagrada or its various extracts have been credited with being among the most suitable of the stimulant laxatives showing a minimum incidence of griping and other side effects (5, 6). A water soluble, bitter free concentrate of the active laxative principles of cascara sagrada was developed by Lee, *et al.*, and this substance, Peristim,² was reported to be approximately ten times as potent as whole cascara sagrada in humans (7).

The purpose of the following study was to verify the relative potency of cascara sagrada and Peristim and to examine some characteristics of Peristim, in mice and rats, in an effort to gain information indicative of its utility as an adjunct to Colace therapy in humans. Danthron, N. F. (1,8-dihydroxyanthraquinone, chrysazin) was similarly investigated.

EXPERIMENTAL

Materials.—Peristim and its fractions, the isopropanol soluble Casanthranol B and the isopro-

panol insoluble residue Casanthranol A, were supplied by S. B. Peniek and Company. Danthron was in the form of the mieronized powder. Peristim and Casanthranol fractions A and B were administered orally as solutions in distilled water. Cascara sagrada extract³ and danthron were suspended in 0.5% methylcellulose 400. The suspensions were passed through a hand homogenizer several times before use and stirred vigorously before each dose. Colace was administered as an aqueous solution and it was freely miscible with all agents; in fact, it improved the consistency of the cascara and danthron suspensions. In the synergism experiments Colace was added to the Peristim solution or danthron suspension. The charcoal mix employed in the rat intestinal-propulsion tests was 6% charcoal in 1% methylcellulose 400.

The influence of several drugs upon the peristaltic-stimulating actions or fecal-hydrating properties was determined. The following drugs were chosen because of their ability to block histamine or various levels of the autonomic nervous system: atropine sulfate, chlorisondamine, phenoxybenzamine, meprobamate, and pyrilamine maleate. All these blocking agents were administered subcutaneously and, with the exception of meprobamate, were dissolved in normal saline. Meprobamate was dissolved in 50% polyethylene glycol 400 and normal saline. The albino mice were all males of the Swiss Webster strain weighing between 18 and 25 Gm. Male and female rats of the Hamilton laboratory stock (Wistar and Carworth Farm) weighing between 150 and 250 Gm. were employed.

Peristaltic-Stimulation Methods.—Propulsive rates of a charcoal mix were determined by the method of Macht (8). The charcoal mix was administered via stomach tube in a volume of 5 cc./Kg. Preliminary experiments revealed that the maximum peristaltic-stimulating effect of either cascara sagrada or Peristim occurred at three to

* Received August 29, 1957, from the Mead Johnson Research Laboratories, Evansville, Ind.

¹ Mead Johnson & Company trade name for dioctyl sodium sulfosuccinate.

² Mead Johnson & Company trade name for a methanol extract of cascara sagrada bark.

³ Cascara sagrada extract N. F. used throughout this study.

four hours following oral administration. Therefore, the charcoal mix was administered three hours and fifteen minutes after the drug and the rats were sacrificed forty-five minutes later. Control animals received the vehicle alone.

Peristim and its fractions (Casanthranol A and Casanthranol B) and cascara sagrada extract were compared for their propulsion stimulating effects by using a self contained 4 point assay in which there were 30 rats at each dosage level. Peristim was arbitrarily chosen as the standard. The results of the assay were evaluated as outlined by Smith and Vos (9) and Knudsen (10).

The effect of Peristim on the rate of stomach emptying during the interval of three to five hours following its administration was determined by a modification of the method of Reynell (11). Three hours after Peristim administration a solution of phenol red, 5 cc/Kg, was given via stomach tube and the homogenized stomach and contents analyzed for the dye at 0, 30, 90 and 180 minutes. The concentration of dye at zero time less the concentration at the appropriate interval was expressed as per cent of stomach emptying.

Postoperative intestinal stasis, as defined by a decreased propulsive rate of charcoal mix, was induced by opening the abdominal cavity under ether anesthesia and manipulating the contents in the same fashion in all rats. The contribution of the operative procedure toward the decreased propulsive rates observed was not determined. Ether anesthesia alone was found to induce similar effects.

Fecal-Hydration Methods—The technique utilizing an "all or none" response essentially as described by Miller (12) for mice was used in both mice and rats. Certain modifications suggested by Lou (13) were adopted as follows: (a) the animals were observed for a period of two hours prior to dosing and those producing abnormally wet feces were discarded; (b) during the test observation period the animals were allowed free access to a special food consisting of equal parts of laboratory chow and tap water. Other modifications adopted involved the withdrawal of food, but not the water, three hours and eighteen hours before dosing the mice and rats, respectively. An attempt was made to achieve uniform hydration of the animals by adjusting the concentration of the fecal hydrating agent so that upon dosing each animal received a water load, 30 cc/Kg for the mice and 20 cc/Kg for the rats. As described by Miller (12) observations were made at appropriate hourly intervals and consisted merely of recording whether or not the feces of the mouse or rat were sufficiently moist to stain the absorbent paper inserted between the wide mesh bottom of each individual cage. Evaluations were made by the method of Litchfield and Wilcoxon (14). In all cases in which an "effective dose₅₀" (ED₅₀) was determined, there were 20 animals at each dosage level and at least 60 animals in each determination.

As a means of demonstrating not only the potency but the speed of onset of the fecal wetting action, dose response curves were drawn and ED₅₀ values determined, from the same mice, at the two-hour, five hour, and eight-hour periods, (Table II).

In some experiments the abdominal cavity of rats was opened under ether or pentobarbital anesthesia and Colace was deposited, via hypodermic syringe, at various levels of the G I tract. Such rats were

returned to their individual cages above the absorbent paper and the feces were examined in the usual manner.

RESULTS

Peristaltic-Stimulating Effects—Rats.—In eleven groups of 20-30 rats each, it was found that controls invariably propelled the charcoal mix so that the farthest point was a mean distance of $61 \pm 3\%$ of the total length of the intestine. When the stimulated peristalsis produced by the drug caused the farthest point of charcoal to lie between the 65% and 85% level, there was an approximately linear log dose response relationship. The cecum, which falls at about the 90% level, was a block to charcoal movement and larger doses of drug would simply increase the percentage of animals in which the charcoal had been propelled to the cecum.

The results of the assay, presented in Table I, show that cascara sagrada extract was about $\frac{1}{3}$ as potent as Peristim or fraction A. The possibility was considered that the lower activity of fraction B might be due to the absence of some suspected synergistic substance which was possibly present in fraction A. This was not the case since combining the two fractions yielded a mixture possessing only the algebraic sum of the two and not the activity of Peristim. The increased propulsive rates seen in Peristim-treated rats were not due to an increased rate of stomach emptying for tests showed that such animals emptied phenol red from their stomachs at a slower rate than did controls.

Figure 1 shows graphically the ability of Peristim to overcome postoperative intestinal stasis and antagonize the propulsion inhibiting effects of a

TABLE I—COMPARATIVE ABILITY OF CASCARA SAGRADA EXTRACTS AND DANTHRON TO STIMULATE THE PROPULSIVE RATE OF A CHARCOAL MIXTURE IN RATS

Laxative Agent	Oral Dose mg/Kg	Mean % of Travel	Relative Potency % \pm Std Error
Controls ^a	Vehicle only	59.4 \pm 1.52	
Peristim	220	70.8	
	480	78.5	100
Cascara sagrada extract N F	600	70.8	37 \pm 3.3
	1310	79.1	
Casanthranol A	220	69.5	
	480	82.0	108 \pm 6.5
Casanthranol B	480	64.0	
	1050	77.3	34 \pm 2.1
Mixture 50% Casanthranol A & 50% Casanthranol B	220	67.2	80 \pm 4.6
	480	77.3	
Danthron ^b	120	58.3 \pm 1.94	
	240	63.5 \pm 1.7	0
	480	63.2 \pm 2.5	
	960	62.3 \pm 1.3	

^a Mean \pm std. error of 60 animals
^b Mean \pm std. error of 20 animals

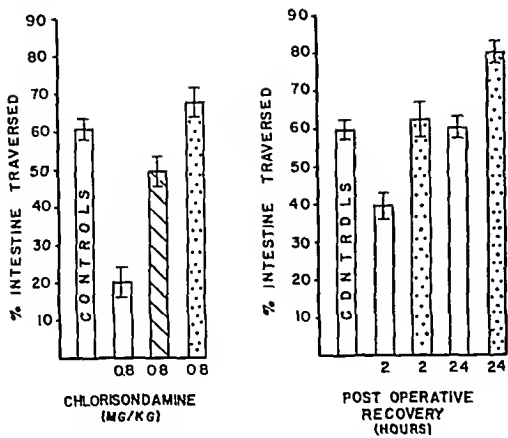


Fig. 1.—Effect of Peristim 240 mg./Kg. (striped area) and 480 mg./Kg. (dotted area) on the decreased propulsive rates induced by a ganglionic blocking agent or abdominal operation under ether anesthesia. Vertical bars indicate standard error of the mean; 20 rats per group.

ganglionic blocking agent. Peristim, in appropriate dosage, was able to decrease or completely overcome the inhibiting effect of chlorisondamine. The stimulating effects of Peristim were therefore considered to be largely independent of functioning intestinal reflexes. Propulsive rates were measured in the danthron-treated rats at intervals varying from forty-five minutes to six hours following drug administration, but there was no evidence of peristaltic stimulation with doses as high as 900 mg./Kg. Colace in doses of 20 mg./Kg. to 100 mg./Kg. showed no significant effect upon propulsive rates. Colace at 200 to 400 mg./Kg. tended to inhibit propulsive rates.

Fecal Hydration—Mice.—A comparison of the ability of six agents to cause excretion of wet feces in mice is shown in Table II. In this test, as in the rat peristaltic-stimulating test, cascara sagrada extract was approximately 1/3 as potent as Peristim. The slower onset of action of the cascara extracts did not permit calculation of an ED₅₀ at the two-hour period following drug administration. The onset of action of danthron was somewhat more rapid than that of the cascara extracts but less rapid than that of Colace. Colace was about nine times as potent as cascara sagrada extract and three and one-half times as potent as Peristim. Only in the case

of danthron did a dose response curve, at the eight-hour test period, deviate significantly (19/20 probability) from parallelism with the Peristim curve. Comparison of the ED₅₀ of these two agents was therefore less meaningful. Between the levels of 40% and 90% effectiveness, danthron was respectively two and one-half times more potent and two and one-fifth times less potent than Peristim.

In an experiment designed to detect addition of drug action (Fig. 2), the dose-effect curve for Peristim obtained from mice treated with 1/2 the ED₅₀ of Colace (0.5 Gm./Kg.) had essentially the same slope as that in mice not receiving Colace. A reasonable criterion for addition of effects was therefore that 1/2 the dose of Peristim required to produce an 80% effect in normal mice should produce the full 80% effect in such Colace-treated mice. Figure 2 demonstrates that this was the case. The ED₅₀ expected for danthron in identical experiments was 3.1 Gm./Kg. but that found was greater than 8.0

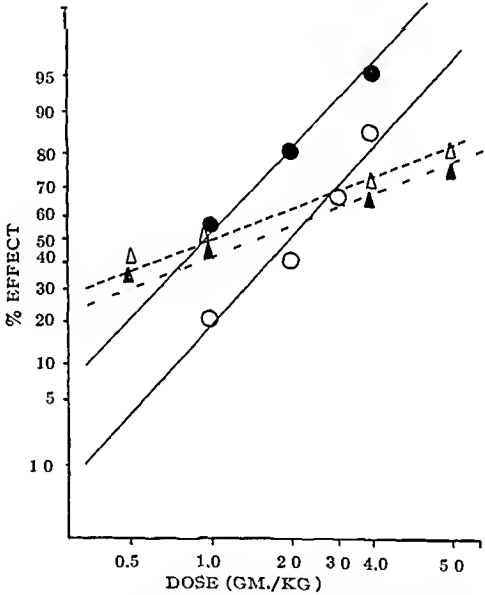


Fig. 2—Mouse fecal-hydrating test, eight-hour test period. Dose effect lines of: Peristim in normal mice ○—○; Peristim in Colace-treated mice ●—●; danthron in normal mice △—△; danthron in Colace-treated mice ▲—▲.

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Drug	ED ₅₀ Gm /Kg (19/20 Confidence Limit)			Slope Function "S," 8 Hr.	Potency Ratio Peristim = 1 (19/20 Confidence limits), 8 Hr.
	2 Hr.	Time Post Drug Administration 5 Hr	8 Hr.		
Peristim	>4 0	2 26 (1 9-2 69)	2 10 (1 6-2 7)	2 0	1 0
Casanthranol A	>4 0	1 76 (1 38-2 25)	1 20 (0 98-1 46)	2 3	1 75 (1.27-2.42)
Casanthranol B	>4 0	1 7 (1 13-2 55)	1 10 (0 97-1 .24)	1 7	1 91 (1.44-2.54)
Cascara sagrada	>12.0	5 2 (3 9-7 0)	5 2 (3 9-7 0)	3 2	0.41 (0.29-0.59)
Danthron	5 0 (3.1-8 0)	1 6 (1 1-2 4)	1 2 (0 6-2 4)	7 3	1.75 (0.74-3.64)
Colace	0.61 (0 47-0.8)	0 59 (0 45-0 77)	0 59 (0.45-0.77)	1 8	3.56 (2.6-5.0)

four hours following oral administration. Therefore, the charcoal mix was administered three hours and fifteen minutes after the drug and the rats were sacrificed forty five minutes later. Control animals received the vehicle alone.

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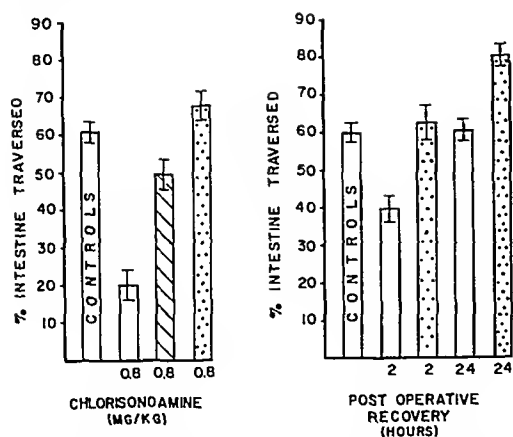


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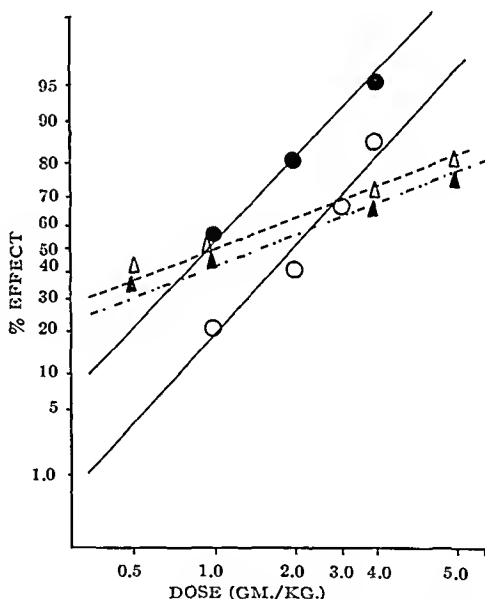


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	2 Hr.	Time Post Drug Administration 5 Hr.	8 Hr.		
Peristim	>4.0	2.26 (1.9–2.69)	2.10 (1.6–2.7)	2.0	1.0
Casanthranol A	>4.0	1.76 (1.38–2.25)	1.20 (0.98–1.46)	2.3	1.75 (1.27–2.42)
Casanthranol B	>4.0	1.7 (1.13–2.55)	1.10 (0.97–1.24)	1.7	1.91 (1.44–2.54)
Cascara sagrada	>12.0	5.2 (3.9–7.0)	5.2 (3.9–7.0)	3.2	0.41 (0.29–0.59)
Danthron	5.0 (3.1–8.0)	1.6 (1.1–2.4)	1.2 (0.6–2.4)	7.3	1.75 (0.74–3.64)
Colace	0.61 (0.47–0.8)	0.59 (0.45–0.77)	0.59 (0.45–0.77)	1.8	3.56 (2.6–5.0)

Gm./Kg. Although Colace depressed dantlron activity at all dose levels, the depression was not statistically significant.

Ganglionic blockade influenced the ED₅₀ of the agents as shown in Table III. The onset of positive responses was not necessarily delayed in the chlorisondamine-treated mice but fewer animals

TABLE III.—EFFECT OF SUBCUTANEOUS CHLORISON-DAMINE ADMINISTRATION^a ON THE FECAL-HYDRATING EFFECTS OF SEVERAL AGENTS IN MICE

Drug	8 Hr. ED ₅₀ with chlorisondamine,
	ED ₅₀ without chlorisondamine
Peristim	1 7
Casanthranol A	1 7
Casanthranol B	1.8
Danthron	>6.0
Colace	>6.0

^a Chlorisondamine administered, in a dose of 0.8 mg./Kg. one hour after oral administration of fecal-hydrating agent.

responded. The highest dose of danthron used, approximately six times the ED₅₀, was effective in only 40% of the animals. Colace was completely without effect, even after twenty-two hours, in doses as high as six times the ED₅₀. Chlorisondamine-inhibition of Peristim was mild suggesting that the ability of Peristim to induce fecal-hydration was largely independent of nervous reflexes. Data in Table IV demonstrate that atropine was also incapable of altering the fecal-hydrating action of Peristim.

With regard to the fecal-hydrating action of Colace, the blocking effect of atropine or chlorisondamine was very evident (Table IV). The effect of the adrenergic blocking agent was weak and questionable. The dose of meprobamate was re-

sponsible for severe and prolonged paralytic symptoms in the mice, but the animals were capable of righting themselves at all times. The blockade of the fecal-hydrating activity of Colace appears to be at the eholincrgic smooth muscle receptor sites or the parasympathetic ganglia of the enteric plexus. The hemodynamic effects produced by the sympathetic ganglion blocking action of chlorisondamine would not appear to be essential for blockade of Colace action.

Fecal-Hydration—Rats. — The fecal-hydrating effects of Colace in normal, bile duct-cannulated, and chlorisondamine-treated rats are shown in Table V. Colace was effective in rats in doses about 1/3 as small as those required in mice.

In the rats, as in the mice, chlorisondamine was a potent inhibitor of Colace activity. This ganglionic blocking agent could prevent or greatly delay the effects of Colace even when the latter was deposited in the proximal colon. That a continual source of bile was not necessary for Colace activity was shown in the rats with bile duct cannulae.

An experiment was conducted in which rats received either with or without a standard dose of Colace, dried whole ox bile, pancreatin, or a combination of bile and pancreatin. These substances did not alter the response of the rats to Colace and had no apparent effect when administered alone.

The results of the fecal-hydration studies of Peristim in normal rats appear in Table VI. The attainment of a near maximal response in rats was somewhat slower than in mice but Peristim was about 10 times more effective in rats than in mice.

DISCUSSION

The fecal-hydration test in the mice and the peristaltic-stimulation studies in rats show Peristim to be 2.5 to 3 times as potent as cascara sagrada extract. Since one gram of the extract represents three grams of the whole bark, the experimental results agree well with the clinical impressions (7) that this sub-

TABLE IV.—EFFECT OF VARIOUS AGENTS ON THE FECAL-HYDRATING ACTION OF PERISTIM AND COLACE IN THE MOUSE

Fecal-Hydrating Agent	Drug	mg./Kg.	Time of Administration ^a (Minutes)	—Positive Response/No. Tested—			
			Postfecal-Hydrating Agent)	1 Hr.	5 Hr.	8 Hr.	22 Hr.
Peristim, 4.0 Gm./Kg. Oral	H ₂ O controls	30 ml.	60 (10 ml./Kg.)	0/20	17/20	20/20	20/20
			120 (10 ml./Kg.)				
			180 (10 ml./Kg.)				
	Atropine sulfate	12	60 (4 mg./Kg.)	0/10	8/10	9/10	9/10
			120 (4 mg./Kg.)				
			180 (4 mg./Kg.)				
Colace, 1.0 Gm./Kg. Oral	Atropine sulfate	36	60 (12 mg./Kg.)	0/10	8/10	10/10	10/10
			120 (12 mg./Kg.)				
			180 (12 mg./Kg.)				
	H ₂ O controls	10 ml.	10	6/10	7/10	7/10	7/10
			10	3/10	3/10	3/10	5/10
			10	0/10	0/10	1/10	2/10
	Chlorisondamine	0.1	10	3/10	3/10	3/10	5/10
			10	0/10	0/10	0/10	0/10
			10 ^a	8/10	8/10	8/10	8/10
	Phenoxybenzamine	10.0	10 ^a	2/10	5/10	5/10	5/10
			10	1/10	3/10	3/10	3/10
			10	9/10	9/10	9/10	9/10

^a Phenoxybenzamine administered 10 minutes before Colace.

stance was about 10 times as potent as whole *cascara sagrada*.

Although Peristim and danthron appear to have approximately equal ability to produce soft hydrated stools in the mouse, this work clearly indicates that the actions of the two substances are not identical. The dose-response curves markedly deviate from parallelism in the mouse fecal-hydration test; danthron lacks the peristaltic-stimulating effects of Peristim in the rat, and danthron is much more susceptible than Peristim to inhibition by ganglionic block in the mouse.

lionic block or intensive anticholinergic treatment. The results suggest that, in the human, Peristim would be a reliable peristaltic-stimulant even in cases of hypomotility induced by operative procedures, anticholinergic, antispasmodic, or hypotensive therapy.

Colace was demonstrated to be a very effective fecal-hydrating agent in both the mouse and rat. Its action was susceptible to inhibition and the results suggest that, in the human, natural or induced conditions associated with decreased intestinal activity and autonomic neuronal transmission may interfere

TABLE V.—FECAL-HYDRATING ACTION OF COLACE ADMINISTERED AT VARIOUS LEVELS OF THE G.I. TRACT OF THE RAT; THE EFFECT OF BILE DUCT CANNULATION AND GANGLIONIC BLOCK

Condition of Rats ^a	Site of Administration of Colace ^b	Positive Response No. Treated (Hours Post Colace Administration) —				
		1	3	5	8	22
Normal	Oral 25 mg./Kg.	0/20	1/20	1/20	3/20	...
Normal	Oral 50 mg./Kg.	0/20	1/20	3/20	5/20	
Normal	Oral 100 mg./Kg.	0/20	7/20	12/20	13/20	
Normal	Oral 200 mg./Kg.	2/20	18/20	18/20	19/20	
Normal	Jejunum	9/10		9/10		9/10
Normal	Jejunum (water only)	0/10		0/10		0/10
Bile duct cannulated (pentobarbital anesthesia)	Jejunum	6/10		10/10		10/10
Chlorisondamine treated ^c	Jejunum	0/10		0/10	0/10	9/10
Normal	Proximal colon	6/8		8/8		8/8
Normal	Proximal colon (water only)	0/9		0/9		0/9
Chlorisondamine treated ^c	Proximal colon	0/10		0/10	0/10	10/10

^a Exclusive of operation necessary for Colace administration, this was always done under ether anesthesia unless otherwise specified.

^b Colace given in dose of 0.2 Gm./Kg. unless otherwise specified.

^c Subcutaneous administration of 0.8 mg./Kg. at the time of Colace administration.

TABLE VI.—FECAL-HYDRATION TEST IN RATS, EFFECT OF PERISTIM

Oral Dose Peristim, Gm./Kg.	Positive Response/ Total Rats			8 hr ED ₅₀ , Gm./Kg.
	Hr Post	Peristim	Admin	
0.125	2/20	3/20	8/20	0.175
0.25	1/20	5/20	12/20	
0.5	3/20	8/20	17/20	
1.5	7/20	14/20	19/20	

The peristaltic-stimulating effects and stool softening effects of Peristim were very dependable. Peristim was shown to counteract the peristaltic-inhibiting effects of chlorisondamine and abdominal surgery under ether anesthesia. Large amounts of atropine were incapable of significantly altering the fecal-hydrating effects of Peristim in mice. It appears that there are at least two components in the total action of Peristim: (a) a reflex independent peristaltic-stimulating effect and, (b) a fecal-hydrating action which proceeds in spite of gang-

lion with its effectiveness. In such cases the addition of a drug with the properties of Peristim would appear to be indicated. The usefulness of danthron as an additive agent would seem limited since it lacks the peristaltic-stimulating effects of Peristim and, like Colace, its fecal-hydrating effects are inhibited strongly by a ganglionic blocking agent.

REFERENCES

- (1) Wilson, J. L., and Dickinson, D. G., *J. Am. Med. Assoc.*, 158, 261 (1955).
- (2) Antos, R. J., *Southwestern Med.*, 37, 236 (1956).
- (3) Kautz, H. D., *J. Am. Med. Assoc.*, 161, 65 (1956).
- (4) Turell, R., *A. M. A. Arch. of Surg.*, 74, 610 (1957).
- (5) Travell, J., *Ann. N. Y. Acad. Sci.*, 58, 416 (1954).
- (6) Goodman, L. S., and Gilman, A., "The Pharmacological Basis of Therapeutics," 2nd ed., The Macmillan Co., New York, 1955, p. 1050.
- (7) Lee, J., *et al.*, U. S. pat. 2,532,896, assigned to Hoffman-La Roche.
- (8) Macht, D. I., *et al.*, *THIS JOURNAL*, 20, 558 (1931).
- (9) Smith, *et al.*, *J. Pharmacol. Exptl. Therap.*, 78, 72 (1943).
- (10) Knudsen, *ibid.*, 86, 339 (1946).
- (11) Reynell, P. C., *et al.*, *J. Physiol.*, 131, 452 (1956).
- (12) Miller, L. C., *et al.*, *THIS JOURNAL*, 38, 417 (1919).
- (13) Lou, T. C., *J. Pharm. and Pharmacol.*, 1, 673 (1949).
- (14) Litchfield, J. T., Jr., and Wilcoxon, R., *J. Pharmacol. Exptl. Therap.*, 96, 99 (1949).

Investigation of Drug Release from Solids II.*

Theoretical and Experimental Study of Influences of Bases and Buffers on Rates of Dissolution of Acidic Solids

By W. I. HIGUCHI, EUGENE L. PARROTT,† J. DALE E. WURSTER, and T. HIGUCHI

A theory is presented which describes the rate of dissolution of solids in basic and buffered media. The formulation is based on the Nernst-Brünner film model assuming a solution diffusion controlled process. Useful expressions are obtained which give relative dissolution rates as functions of the primary variables. Results of calculations predict that for a solid weak acid dissolving in an aqueous basic solution, the rates of dissolution become essentially independent of the strength of the base above certain values. In the region where it is independent of the base strength the rate is a linear function of the product of the base concentration and the base diffusion coefficient. The theoretical conclusions are satisfactorily substantiated by experiments on the rates of dissolution of benzoic acid in basic media of varying strength and diffusivities. The following bases were used as additives: sodium hydroxide, sodium bicarbonate, sodium tetraborate, disodium phosphate, sodium acetate, and ethanolamine. Studies were also carried out with added swamping electrolyte. It was found that the rates of dissolution in varying concentrations of sodium chloride in neutral solutions can be largely explained by the salting out effects of the electrolyte.

IT IS OF IMPORTANCE both to the manufacturer and the administrator of pharmaceutical dosage forms to clearly understand the fundamental factors which influence the rate of dissolution of drugs. The kinetics problem of a substance dissolving in a nonreacting medium is relatively well understood (1-6). As physiological conditions are, more often than not, somewhat removed from neutrality, it is highly desirable to establish the primary effects which basicity and acidity induce on the dissolution rates of an acidic or basic substance. Furthermore, it is desirable to examine the influence of a neutral salt upon the dissolution rates in both neutral and reactive media.

King and Brodie (7) and Hixon and Baum (8) have reported results on the dissolution rates of benzoic acid in dilute aqueous sodium hydroxide and potassium hydroxide. They have satisfactorily explained their data on the basis of the Nernst-Brünner two film model of diffusion controlled kinetics. Their theory can be described as shown in Fig. 1. It is assumed that there exists an effective liquid film between the surface of the benzoic acid solid and the stirred bulk solution. At the solid-film interface the film is saturated with benzoic acid. The diffusion of benzoic acid through this film of thickness $x + y$ is solely responsible for the rate of dissolution. Linear concentration gradients of all species as shown in

Fig. 1 are assumed to exist in this film. The rate of dissolution then is proportional to the slope of the (HA) line.

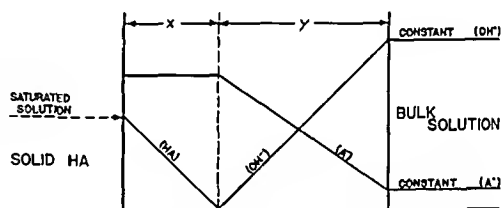


Fig. 1.—Concentration profiles during the dissolution of benzoic acid in aqueous sodium or potassium hydroxide solutions. The film thickness is $x + y$.

It is clear that this picture neglects the effects of the base strength of the medium and the diffusivities of the reaction products. Moreover, it assumes that all of the reaction occurs at the plane where the reactants "meet," disregarding the possibility of neutralization elsewhere. Although such a model explains well systems involving a reaction equilibrium which lies very far to the right, it is expected to fail for the general case. It is one of the intents of this research to provide a general theory, substantiated by experiments, which includes the influence of the equilibrium constants among the factors considered.

The studies reported here are limited to the kinetics of the process: Weak Acid (solid) \rightarrow Solution. The general conclusions however may be extended and applied to any problem in which the process is diffusion controlled in the solution phase.

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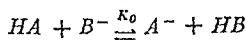
The paper is based on a dissertation submitted by Eugene L. Parrott to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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THEORY

The formulation is based on the Nerst-Brünner model (9) of a diffusion controlled process in the solution phase. It is assumed that the effective liquid film concept of representing mass transfer is applicable. The thickness of this film is assumed to be independent of the diffusivities at constant stirring rates, viscosity, and geometry of the system, and the diffusion coefficients are assumed to be independent of concentration. Furthermore, all chemical equilibria are assumed to be rapid compared to diffusion.

For the problem of the rate of dissolution of a solid weak acid, HA , in a solution of an ionic base B^- the important chemical equilibrium is



where as usual

$$K_0 = \frac{K_{HA}}{K_{HB}} = \frac{(A^-)(HB)}{(HA)(B^-)} \frac{\gamma_{A^-}\gamma_{HB}}{\gamma_{HA}\gamma_{B^-}}$$

is the thermodynamic equilibrium constant. (S) is the concentration of species S and γ_S is the activity coefficient of S . The function K

$$K = K_0 \frac{\gamma_{HA}\gamma_{B^-}}{\gamma_{A^-}\gamma_{HB}} \frac{(A^-)(HA)}{(HA)(B^-)} \quad (\text{Eq. 1})$$

then is the concentration equilibrium constant.

Fig. 2 describes qualitatively the steady state situation.

At $X = 0$, $(HA) = (HA)_0$, $(HB) = (HB)_0$, $(A^-) = (A^-)_0$, and $(B^-) = (B^-)_0$. (Eq. 2A)

At $X = h$, $(HA) = (HA)_h$, $(HB) = (HB)_h$, $(A^-) = (A^-)_h$, and $(B^-) = (B^-)_h$. (Eq. 2B)

For the steady state case of the one dimensional problem, the application of Fick's law (10) leads to the following set of equations:

$$\frac{d(HA)}{dt} = D_{HA} \frac{d^2(HA)}{dx^2} + \phi_1 = 0$$

$$\frac{d(HB)}{dt} = D_{HB} \frac{d^2(HB)}{dx^2} + \phi_2 = 0$$

$$\frac{d(A^-)}{dt} = D_{A^-} \frac{d^2(A^-)}{dx^2} + \phi_3 = 0$$

$$\frac{d(B^-)}{dt} = D_{B^-} \frac{d^2(B^-)}{dx^2} + \phi_4 = 0$$

D_S is the diffusion coefficient of species S in the system. The ϕ 's are the source or sink functions. For proper material balance

$$D_{HA} \frac{d^2(HA)}{dx^2} = -D_{HB} \frac{d^2(HB)}{dx^2} = -D_{A^-} \frac{d^2(A^-)}{dx^2} = D_{B^-} \frac{d^2(B^-)}{dx^2} \quad (\text{Eq. 3})$$

$$-D_{B^-}(B^-) = \frac{D_{B^-}(L_1 + L_2) + D_{HB}D_{A^-}K(HA) \pm \{[D_{B^-}(L_1 + L_2) + D_{HB}D_{A^-}K(HA)]^2 - 4L_1L_2D_{B^-}^2\}^{1/2}}{2D_{B^-}}$$

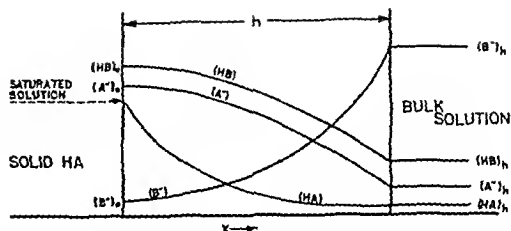


Fig. 2.—Concentration profiles during the dissolution of a weak acid, HA , in aqueous ionic base. The film thickness is h and B^- is the base ion.

Hence $\phi_1 = \phi_2 = \phi_3 = \phi_4 = 0$. Equation 3 means that for a given amount of reaction of HA and B^- in any volume element, equivalent amounts of HB and A^- are produced. ϕ then is the rate of neutralization per unit volume.

The solutions to Eq. 3 obtained by integration are

$$D_{HA} \frac{d(HA)}{dx} + C_1 = -D_{HB} \frac{d(HB)}{dx} + C_2 = -D_{A^-} \frac{d(A^-)}{dx} + C_3 = D_{B^-} \frac{d(B^-)}{dx} \quad (\text{Eq. 4})$$

where the C 's are the integration constants. The boundary conditions at $x = h$ are by material balance.

$$-D_{B^-} \frac{d(B^-)}{dx} = D_{A^-} \frac{d(A^-)}{dx} = D_{HB} \frac{d(HB)}{dx} \quad (\text{Eq. 5})$$

Therefore,

$$C_2 = C_3 = 0$$

Also by material balance it is evident that

$$C_1 = \left(D_{B^-} \frac{d(B^-)}{dx} \right)_{x=h} - \left(D_{HA} \frac{d(HA)}{dx} \right)_{x=h} = \left(D_{HA} \frac{d(HA)}{dx} \right)_{x=0} \quad (\text{Eq. 6})$$

Therefore, C_1 is the rate of dissolution of HA per unit area of the film.

Now the integration of Eq. 4 with $C_2 = C_3 = 0$ yields

$$D_{B^-}(B^-) = -D_{A^-}(A^-) + L_1 \quad (\text{Eq. 7})$$

$$D_{B^-}(B^-) = -D_{HB}(HB) + L_2 \quad (\text{Eq. 8})$$

$$D_{B^-}(B^-) = D_{HA}(HA) + C_1X + L_3 \quad (\text{Eq. 9})$$

where L_1 , L_2 , and L_3 are integration constants.

By applying the boundary conditions Eq. 2b L_1 and L_2 are evaluated:

$$L_1 = D_{B^-}(B^-)_h + D_{A^-}(A^-)_h$$

and

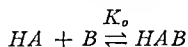
$$L_2 = D_{B^-}(B^-)_h + D_{HB}(HB)_h$$

Combining Eq. 7, Eq. 8, and Eq. 1 and solving for $D_{B^-}(B^-)$ one obtains

which when combined with Eq 9 at $X = 0$ yields the expression for L_3 . Now taking this expression for L_3 and solving for C_1 employing Eq 9 at $X = h$ results in the final expression

$$C_1 = \frac{D_B(B^-)_h - D_{HA}(HA)_h + D_{HA}(HA)_o}{h} - \frac{D_B(L_1 + L_2) + D_{HB}D_A K(HA)_o \pm \{[D_B(L_1 + L_2) + D_{HB}D_A K(HA)_o]^2 - 4L_1L_2D_B^{-2}\}^{1/2}}{2D_B^{-h}} \quad (\text{Eq 10})$$

In a similar manner the problem may be solved for the analogous situation involving the nonionic equilibrium



where similarly

$$K_o = \frac{(HAB)\gamma_{HAB}}{(HA)(B)\gamma_{HA}\gamma_B} = \frac{\bar{K}\gamma_{HAB}}{\gamma_{HA}\gamma_B} \quad (\text{Eq 11})$$

The rate of dissolution per unit area of a solid HA in a solution of the base B may be shown to be

$$C_1 = \frac{D_{HA}(HA)_o - D_{HA}(HA)_h}{h} + \frac{D_B W}{h} \left(\frac{1}{(HA)_o D_{HAB} \bar{K} + D_B} - \frac{1}{(HA)_h D_{HAB} \bar{K} + D_B} \right) \quad (\text{Eq 12})$$

where W is a constant

$$W = -D_{HAB}(HAB)_h - D_B(B)_h$$

and \bar{K} is defined by Eq 11

When the base may react with two or more acid molecules or when more than one base is present, the problem becomes somewhat more algebraically unwieldy but the general treatment is essentially the same as that for the simpler cases. The appendix discusses the problem of HA (solid) dissolving in the presence of a diacidic base.

It can easily be shown that Eq 10 and 12 reduce to their expected expressions in the limits

$$K \rightarrow 0$$

and

$$K \rightarrow \infty$$

Where K is very small both Eq 10 and 12 is approximated by

$$C_1 = \frac{D_{HA}(HA)_o - D_{HA}(HA)_h}{h} \quad (\text{Eq 13})$$

Where K is very large Eq 10 and 12 reduce to

$$C_1 = \frac{D_{HA}(HA)_o - D_{HA}(HA)_h + D_B(B^-)_h}{h} \quad (\text{Eq 14})$$

and

$$C_1 = \frac{D_{HA}(HA)_o - D_{HA}(HA)_h + D_B(B)_h}{h} \quad (\text{Eq 15})$$

respectively. Equations 14 and 15 predict linear concentration gradients in the film for both acid and the base. These would form the basis of the theory applied by King and Brodie (7) and Hixon and Baum (8) to their data.

Let us now ascertain the K values which would permit the use of Eq 13 or 14 and 15 as sufficient approximations say to about 1% of Eq 10 and 12. Since only approximate magnitudes are desired, all diffusion coefficients are assumed to be equal.

Also taking $(A^-)_h = (HB)_h = (HA)_h = 0$, eq 10 reduces to

$$C_1 = \frac{D(HA)_o}{h} - \frac{DK(HA)_o}{2h} + \frac{DK(HA)_o}{2h} \left(1 + \frac{4(B^-)_h}{K(HA)_o} \right)^{1/2} \quad (\text{Eq 16})$$

Expansion of the square root term to three terms and neglecting the other gives

$$C_1 = \frac{D(HA)_o}{h} + \frac{D(B^-)_h}{h} - \frac{2D(B^-)_h^2}{Kh(HA)_o} \quad (\text{Eq 17})$$

For Eq 14 to approximate Eq 10 to about 1% it is seen that K must be

$$\begin{aligned} K &> 10^2, \text{ if } (HA)_o = (B^-)_h \\ K &> 2 \times 10^3, \text{ if } 10(HA)_o = (B^-)_h \\ K &> 2 \times 10^4, \text{ if } 100(HA)_o = (B^-)_h \end{aligned} \quad (\text{Eq 18})$$

It is found with the aid of eq 16 that for Eq 13 to approximate Eq 10 to about 1% K must be

$$\begin{aligned} K &< 10^{-4} \text{ if } (HA)_o = (B^-)_h \\ K &< 10^{-5} \text{ if } 10(HA)_o = (B^-)_h \\ K &< 10^{-6} \text{ if } 100(HA)_o = (B^-)_h \end{aligned} \quad (\text{Eq 19})$$

Let us now examine eq 12 and decide how large K must be in this case for Eq 15 to approximate Eq 12. Assume again that $D_{HA} = D_B = D_{HAB} = D$ and that $(HAB)_h = (HA)_h = 0$. It is seen from eq 12 that K must be

$$K > \frac{10^2}{(HA)_o} \quad (\text{Eq 20})$$

in order that the approximation be good to 1%. Also for the nonionic base K must be

$$\begin{aligned} K &< 1 \text{ for } (B)_h = (HA)_o \\ K &< 10^{-1} \text{ for } (B)_h = 10(HA)_o \\ K &< 10^{-2} \text{ for } (B)_h = 100(HA)_o \end{aligned} \quad (\text{Eq 21})$$

in order that the base not influence the dissolution rate to more than about 1%.

As a numerical example, ionic bases having a K_B greater than about 10^{-7} will influence the rate of dissolution of benzoic acid ($K_A = 6 \times 10^{-6}$) essentially independently of their strengths. Non-ionic bases having K_B greater than about 10^{-6} will also act independently of their strengths on benzoic acid dissolution rates. The above calculations refer to nonacidic bases, of course.

EXPERIMENTAL

In order to test the theoretical conclusions of the preceding section, experiments were carried out on the rates of dissolution of benzoic acid in the presence of various alkaline additives with and without added inert electrolyte. The choice of the alkali

line substances were based on several factors: desirability of wide ranges of base strengths, types, and diffusivities. The purpose of the added neutral electrolyte was to determine the nature and the magnitudes of the salt effects. It was expected that the electrical effects of the electrolyte would modify the diffusion rates of the ionic species (11).

Apparatus and Procedure.—The apparatus and method have already been described (3). The rates at 25° were determined from both the radius and weight measurements. Two liters of various concentrations of sodium hydroxide, disodium phosphate, sodium bicarbonate, sodium acetate, sodium tetraborate, and ethanolamine were used as solvents with and without 0.75 *M* sodium chloride as an additive. Experiments were also carried out in pure sodium chloride solutions.

RESULTS AND DISCUSSION

The results are summarized in Tables I, II, and III and in Figs 3 to 9.

TABLE I.—RATE OF DISSOLUTION OF BENZOIC ACID IN SODIUM CHLORIDE SOLUTIONS

Rate, Gm. cm. ⁻² Hr. ⁻¹	Conc. NaCl, M/L	Solubility, M/L
0.0426	0.00	0.0285
0.0414	0.154	0.0281
0.030	0.75	0.0203
0.0274	1.008	0.0188
0.0218	1.545	0.0155
0.0102	3.09	0.0082
0.0044	4.635	0.0042

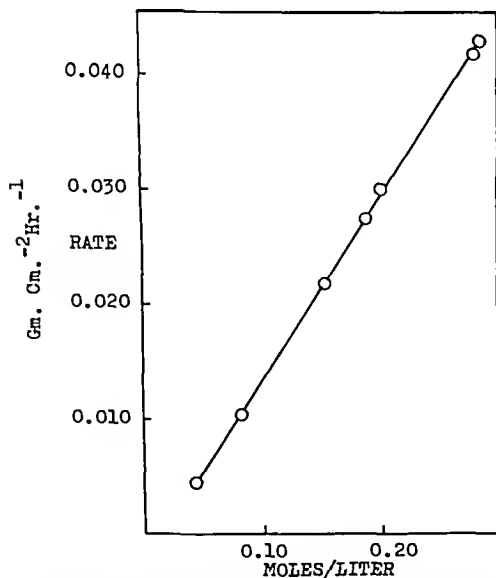


Fig 3—A plot of the dissolution rate vs the solubility of benzoic acid in NaCl solutions of various concentration (See Table I)

Rates in Pure Sodium Chloride Solutions.—For the discussion and later calculations, the value of *h*, the film thickness, is now determined. The dissolution rate of benzoic acid in pure water under the conditions of these experiments is 0.0426 Gm. cm.⁻² hr.⁻¹ (3). The solubility of benzoic acid in pure water is 0.0285 *M*. Taking the value for the diffusion coefficient of benzoic acid in pure water measured by King and Brodie (7), viz. $D_{HA} = 1.11 \times 10^{-5}$ cm.² sec.⁻¹, one obtains, using Eq 13,

$$h = 3.28 \times 10^{-3} \text{ cm.}$$

From the slope of the apparently linear plot of Fig 3 and Eq 13 the diffusion coefficient of benzoic

TABLE II.—RATE OF DISSOLUTION OF BENZOIC ACID TABLETS IN VARIOUS CONCENTRATIONS OF SOME ALKALINE SUBSTANCES

Concentration, Moles/Liter	Rate Radius Method	Weight Method
For NaOH		
0.0125	0.070	0.072
0.0312	0.139	0.135
0.0625	0.250	0.243
0.125	0.455	0.444
0.222	0.732	0.732
0.250		0.871
For CH ₃ COONa		
0.013	0.057	0.059
0.015	0.055	0.056
0.050	0.090	0.092
0.052	0.100	0.096
0.10	0.127	0.130
0.15	0.160	0.158
0.20	0.177	0.183
0.25	0.206	0.204
0.25	0.195	0.204
0.33	0.213	0.227
For Na ₂ HPO ₄		
0.0113	0.058	0.060
0.05	0.107	0.111
0.10	0.167	0.169
0.113	0.188	0.190
0.20	0.273	0.290
0.25	0.344	0.363
For Na ₂ B ₄ O ₇		
0.01	0.069	0.070
0.05	0.179	0.184
0.10	0.315	0.339
0.12	0.336	0.396
0.15	0.443	0.474
For NaHCO ₃		
0.025	0.060	
0.05	0.077	
0.10	0.100	
0.15	0.107	
0.20	0.110	
0.20	0.123	
For Ethanolamine		
0.02		0.070
0.032		0.098
0.05		0.124
0.089		0.184
0.158		0.291
0.206		0.384
0.275		0.471

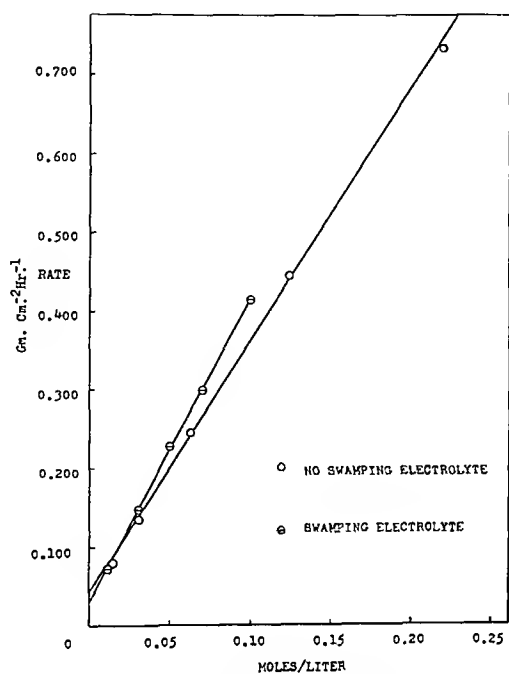


Fig. 4—The rate of dissolution of benzoic acid in sodium hydroxide solutions

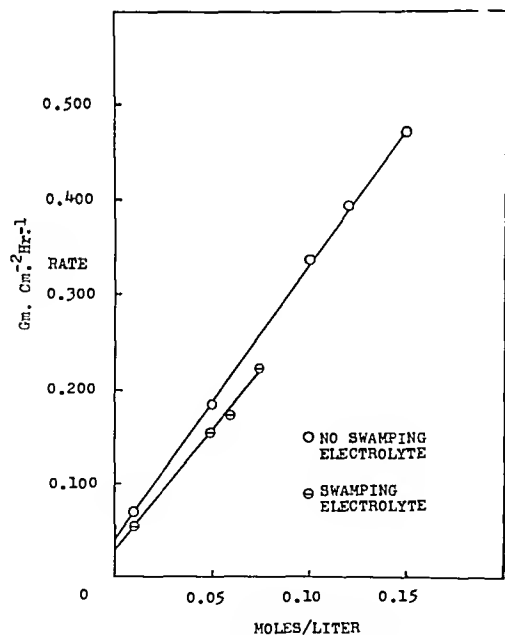


Fig. 5—The rate of dissolution of benzoic acid in sodium tetraborate solutions

acid in sodium chloride solution is obtained, viz. $D_{HA} = 1.16 \times 10^{-6}$ cm.² sec.⁻¹. The linearity of the plot and the relatively close agreement of the diffusion coefficients over a wide range of sodium

chloride concentrations may be ascribed to: the validity of Eq. 13 and the diffusion coefficient of benzoic acid being relatively constant over the range of 0 to 4 M sodium chloride. Since the decrease in solubility is due to the salting-out effect of the elec-

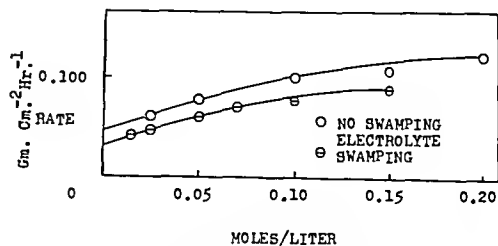


Fig. 6—The rate of dissolution of benzoic acid in sodium bicarbonate solutions.

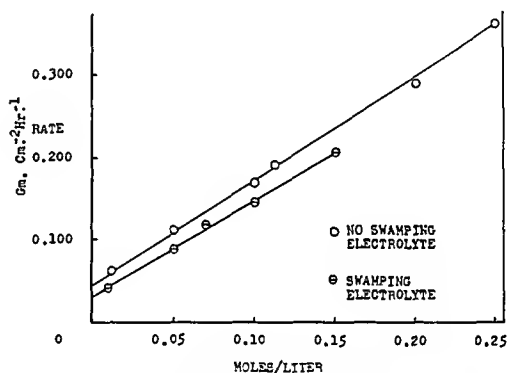


Fig. 7—The rate of dissolution of benzoic acid in disodium phosphate solutions.

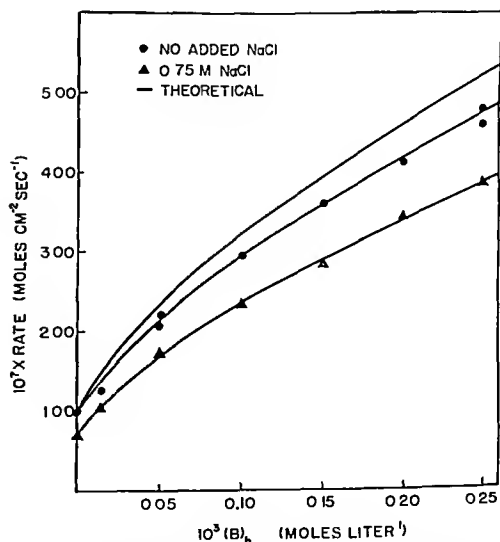


Fig. 8—The dissolution rate of benzoic acid in aqueous sodium acetate $(B)_a$ is the sodium acetate concentration.

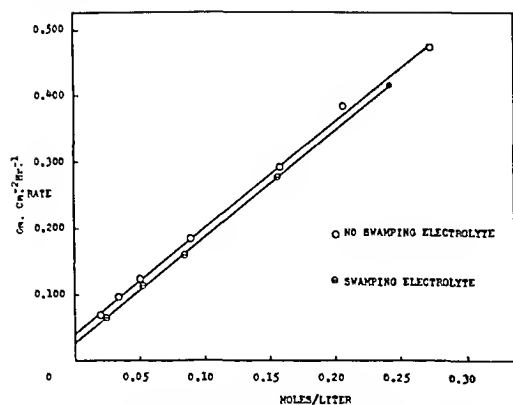


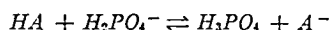
Fig. 9.—The rate of dissolution of benzoic acid in ethanolamine solutions.

TABLE III.—RATE AND CONCENTRATION OF ALKALINE IONIC SUBSTANCE IN THE PRESENCE OF A SWAMPING ELECTROLYTE (0.75 *M* NaCl)

Concentration, Moles/Liter	Rate
For NaOH	
0.015	0.078
0.03	0.147
0.05	0.227
0.07	0.298
0.10	0.413
For CH ₃ COONa	
0.015	0.045
0.05	0.077
0.10	0.103
0.15	0.124
0.20	0.151
0.25	0.168
For Na ₂ HPO ₄	
0.01	0.041
0.05	0.087
0.07	0.118
0.10	0.143
0.15	0.204
For Na ₂ B ₄ O ₇	
0.01	0.055
0.05	0.153
0.06	0.173
0.075	0.222
For Ethanolamine	
0.024	0.066
0.052	0.117
0.085	0.160
0.156	0.278
0.242	0.418

and sodium acetate respectively, all plots are essentially straight lines. For these the linear expressions Eq. 14 or 15 are applicable, i. e., their respective K values are sufficiently great so that the approximate expression suffices. The K values are: 6×10^9 for sodium hydroxide, 1×10^5 for boric acid, 1×10^3 for disodium phosphate (reaction with one hydrogen equivalent), and 2×10^5 for ethanolamine. All meet conditions Eq. 18 or 20. For these substances the rate of dissolution of benzoic acid is uninfluenced by their base strengths.

A calculation will show that the reaction equilibrium



where HA = benzoic acid, is always far to the left throughout the film. As the benzoate ion concentration is relatively large, the ratio $H_3PO_4/H_2PO_4^-$ is nowhere greater than about 1%. Hence for benzoic acid dissolution, under the conditions of these experiments disodium phosphate will act monoacidic. The general case of a weak acid solid dissolving in the presence of a diacid base should be treated according to the method discussed in the appendix.

A tetraborate ion is interpreted as two borate ions (12). Although at the higher base concentrations there may be appreciable tetraborate ions in the stirred solution, probably most of the diffusing species and certainly all of the reacting species are borate ions.

The plots of the rates of dissolution of benzoic acid in sodium bicarbonate solution both with and without a swamping electrolyte indicate (Fig. 6) marked deviations from linearity. As the K for this case is about 200, linearity to better than 10% was predicted by Eq. 17. The deviations are ascribed to carbon dioxide gas pockets which were visually observed at the solid-solution interface, generating an added resistance which varied with the concentration of the bicarbonate. Calculations in the next section confirm this conclusion. A mathematical treatment of a problem of this type is seriously complicated by the nonequilibrium conditions existing at the interface. The surface properties of the solid will determine to a large extent the ease of liberation of the gas, and hence the rate of dissolution.

The nonlinear behavior in the presence of sodium acetate was expected in accordance with the theory. The K value of about 3.7 is too small for Eq. 14 to apply and yet too large for the alkaline effects to be neglected. Hence only the complete expression (10) will apply. The test of (10) for this case is carried out below.

Some deviations from linearity, though generally of much smaller magnitude than those observed with sodium carbonate and sodium acetate, may be expected as consequences of the assumptions underlying the theory even for those systems where $K > 10^3$. The diffusion coefficients may vary with the concentration and hence with distance across the film. They may also vary with the different ionic and nonionic species present at various concentrations across the film. The film thickness has been noted (13, 14, 8) to be not entirely independent of the diffusivities. The salting-in effect of the benzoate ion (15) will to some ex-

trollyte, it can be said that the influence of sodium chloride on the dissolution rate of benzoic acid under these conditions is almost wholly explained by this salting out effect.

Linear and Nonlinear Increase of Rates with Base Concentrations.—Except for Figs. 6 and 8 which are plots of the rates in sodium bicarbonate

tent affect (HA) Nevertheless, all of these effects influence to only a small extent the linearity of a system where $K > 10^3$ as demonstrated by the plots

Quantitative test of theory and the effects of an added electrolyte—Employing Eq 14 or 15 where applicable the diffusion coefficients may be calculated from the experimental results and compared with values in the literature or with values estimated from conductance data Unfortunately the literature data are scanty, and where available, usually pertain to conditions somewhat removed from the situation of the experiments here From the slopes of the straight lines in Figs 4, 5, 6, 7, and 9 the diffusion coefficients are calculated as

$$D_B = \text{slope}/h = \text{slope}/3.3 \times 10^{-2} \text{cm}$$

Table IV, columns two and three give these calculated values For sodium hydroxide the agreement with the limiting value and King and Brodie's value is good In this case the effect of 0.75 M sodium chloride is to increase the diffusion coefficient of the hydroxide ion by about 12%

low bicarbonate concentrations is far from the limiting value This disagreement follows from the observation of gas bubble formation at the interface discussed earlier With ethanalamine there is very little salt effect on the diffusion coefficient It appears that nonionic agents are relatively unaffected by electrolytes, although such a general statement is perhaps dangerous in view of the complex nature of the problem (17, 18) Again, no diffusion data on ethanalamine was found in the literature, but its value appears reasonable when compared to those of some of the primary alcohols tabulated by Jost (10)

The crucial test of the theory is possible with the results obtained with sodium acetate In this case the bracket term of Eq 10 may not be neglected Taking (10) and for

$$D_A^-(A^-)_h = D_{HB}(HB)_h = D_{HA}(HA)_h = 0$$

$$C_1 = \frac{D_{HA}(HA)_0}{h} - \frac{D_{HB}D_A^-K(HA)_0}{2D_B^-h} + \frac{D_{HB}D_A^-K(HA)_0}{2D_B^-h} \left(1 + \frac{4D_B^-(B^-)_h}{D_{HB}D_A^-K(HA)_0} \right)^{1/2}$$

TABLE IV—EXPERIMENTAL DIFFUSION COEFFICIENTS FROM RATES OF DISSOLUTION OF BENZOIC ACID

Substance	D_B^- or D_B No Added Electrolyte	D_B^- or D_B With 0.75 M NaCl	D_B or D_B Literature
Sodium hydroxide	2.42×10^{-5}	2.92×10^{-5}	2.42×10^{-5a} $2.7-2.9 \times 10^{-5b}$
Sodium borate (Sodium tetraborate)	1.03×10^{-5}	0.93×10^{-5}	
Disodium phosphate	0.97×10^{-5}	0.87×10^{-5}	1.06×10^{-5a}
Ethanalamine	1.22×10^{-5}	1.20×10^{-5}	
Sodium bicarbonate	5.0×10^{-6c}	6.4×10^{-6c}	1.25×10^{-5a}

Units of $\text{cm}^2 \text{sec}^{-1}$

^a Using conductance data tabulated in references 11 and 16 and using Nernst's and Haskell's limiting equations (reference 11) for infinite dilution

^b King's recommended value (reference 7)

^c Initial slopes used

This increase is primarily due to the suppression of the diffusion potential (11) created by the sodium ion overwhelming the usual electrolyte depression of the diffusion coefficient of ionic substances as observed in the cases of disodium phosphate and sodium borate This diffusion potential arises when the diffusing ions do not all have about the same mobility It is particularly noticeable in systems involving hydrogen or hydroxide ions (11) and is probably of secondary importance in the cases of disodium phosphate and sodium borate In the latter the diffusion coefficients decrease with added electrolyte as is generally expected (17, 18) With disodium phosphate the agreement with limiting value is satisfactory Here the limiting value is expected to be greater than the observed value in column 1, and by about the amount observed, viz. ~10% (11) Unfortunately no conductance data could be found for sodium borate and hence a check was not possible However, the observed value of 1.03×10^{-5} appears to be reasonable when compared with 0.97×10^{-5} of the disodium phosphate, or with the limiting value of 1.25×10^{-5} for sodium bicarbonate In the case of sodium bicarbonate even the diffusion coefficient obtained at

The following values are employed $K = 3.67$, $(HA)_0 = 0.0285 M$, $h = 3.3 \times 10^{-2} \text{cm}$, $D_{HB} = 1.28 \times 10^{-5} \text{cm}^2 \text{sec}^{-1}$ (extrapolated from values at 0.1 M and 18° and below in reference 19), $D_{B^-} = 1.19 \times 10^{-5}$ (references 11, 16, limiting value), $D_{HA} = 1.11 \times 10^{-5}$, $D_{A^-} = 1.04 \times 10^{-5}$ (reference 11, 16, limiting value)

Then,

$$C_1 = 1.78 \times 10^{-7} (1 + 4.13 \times 10^4 [B^-]_h)^{1/2} - 0.81 \times 10^{-7} \text{moles cm}^{-2} \text{sec}^{-1}$$

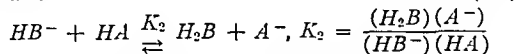
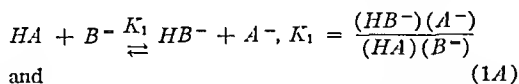
The plot of this equation is given in Fig 8 The agreement must be considered satisfactory recalling that only approximate values for the diffusion coefficients were used In choosing $K = 3.67$ it was assumed that the thermodynamic salt effects cancel in the reaction, and therefore the activity coefficients cancel It is notable that the experimental curve without the swamping electrolyte falls between the theoretical curve and the experimental curve with the added electrolyte This salt effect trend was also observed with disodium phosphate and sodium tetraborate, but reversed in the case of sodium hydroxide for reasons already given It appears safe to state that, except for

bases involving abnormally rapid or slow ions, sodium chloride at 0.75 *M* will decrease the base controlled rates of dissolution of benzoic acid by about 10 to 20% aside from that due to the salting out effects

APPENDIX

Dissolution of Acidic Solids in Presence of Diaacidic Base

For the case of *HA* (solid) dissolving in the presence of a diaacidic base, *B*⁻, the important equilibria are



where *K*₁ and *K*₂ are concentration equilibrium constants. The appropriate differential equations to be solved are

$$D_{HA} \frac{d^2(HA)}{dx^2} = D_{B^-} \frac{d^2(B^-)}{dx^2} - D_{HB^-} \frac{d^2(H_2B)}{dx^2} - D_{A^-} \frac{d^2(A^-)}{dx^2} \quad (2A)$$

and

$$D_{B^-} \frac{d^2(B^-)}{dx^2} = -D_{HB^-} \frac{d^2(HB^-)}{dx^2} - D_{H_2B} \frac{d^2(H_2B)}{dx^2}$$

The solutions to (2A) employing (1A) are

$$D_{B^-}(B^-) = -D_{HB^-} \frac{(HA)(B^-)K_1}{(A^-)} - D_{H_2B}(H_2B) + L_1,$$

$$D_{B^-}(B^-) = -D_{HB^-} \frac{(H_2B)(A^-)}{K_2(HA)} - D_{H_2B}(H_2B) + L_1,$$

$$D_{B^-}(B^-) = D_{H_2B}(H_2B) - D_{A^-}(A^-) + L_2,$$

and

$$D_{B^-}(B^-) + C_1X = D_{HA}(HA) + D_{H_2B}(H_2B) + L_3$$

*L*₁, *L*₂, and *L*₃, and *C*₁ are constants, where *C*₁ is the rate of dissolution of *HA* per unit area as before. *L*₁ and *L*₂ may be taken equal to *D*_{*B*⁻}(*B*⁻)_h if (*HB*⁻)_h = (*H*₂*B*)_h = 0. The problem remaining is

to evaluate *L*₃ and then *C*₁ algebraically. The explicit solution would involve a quartic equation. Therefore the method of successive approximations would be more convenient. The final set of two equations to be solved are

$$\begin{aligned} & \{ (D_{B^-}(B^-) - L_1)^2 - K_1 D_{HB^-}(HA)(B^-)D_{A^-} \}^{1/2} = \\ & - \frac{1}{2D_{HB^-}} (L_2 D_{HB^-} - D_{HB^-} D_{B^-}(B^-) + \\ & K_2 D_{A^-} D_{H_2B}(HA)) + \frac{1}{2D_{HB^-}} \{ (L_2 D_{HB^-} - \\ & D_{HB^-} D_{B^-}(B^-) + K_2 D_{A^-} D_{H_2B}(HA))^2 + \\ & 4L_1 D_{HB^-} D_{H_2B} D_{A^-} (HA) K_2 - \\ & 4K_2 D_{A^-} D_{B^-} D_{HB^-} D_{H_2B}(HA)(B^-) \}^{1/2} \end{aligned}$$

and

$$(B^-) \{ 2D_{B^-}(C_1X - D_{HA}(HA) - L_3 + 2D_{B^-}L_1 + K_1 D_{HB^-} D_{A^-} (HA)) \} = L_1^2 - (C_1X - D_{HA}(HA) - L_3)^2$$

Now these equations may be solved at *x* = 0 and *x* = *h* and *C*₁ evaluated as a function of (*B*⁻)_h, (*HA*)₀, *K*₁, *K*₂, and the diffusion coefficients

REFERENCES

- (1) Noyes, A. A., and Whitney, W. R., *Z. physik. Chem.*, **23**, 689 (1897).
- (2) Noyes, A. A., and Whitney, W. R., *J. Am. Chem. Soc.*, **19**, 930 (1897).
- (3) Parrott, E. L., Wurster, D. E., and Higuchi, T., *This Journal*, **44**, 269 (1955).
- (4) Hixon, A. W., and Crowell, J. H., *Ind. Eng. Chem.*, **23**, 923 (1931).
- (5) Hixon, A. W., and Crowell, J. H., *ibid.*, **23**, 1002 (1931).
- (6) King, C. V., *J. Am. Chem. Soc.*, **57**, 828 (1935).
- (7) King, C. V., and Brodie, S. S., *J. Am. Chem. Soc.*, **59**, 1375 (1937).
- (8) Hixon, A. W., and Baum, S. J., *Ind. Eng. Chem.*, **36**, 528 (1944).
- (9) Brunner, E., *Z. physik. Chem.*, **47**, 56 (1904).
- (10) Jost, W., "Diffusion," Academic Press Inc., New York, 1952, p. 475.
- (11) Vinograd, J. R., and McBain, J. W., *J. Am. Chem. Soc.*, **63**, 2008 (1941).
- (12) Latimer, W. M., "Oxidation Potentials," Prentice-Hall, Inc., New York, 1952.
- (13) King, C. V., *J. Am. Chem. Soc.*, **57**, 828 (1935).
- (14) King, C. V., and Howard, P. L., *Ind. Eng. Chem.*, **29**, 75 (1937).
- (15) Long, F. A., and McDevit, W. F., *Chem. Revs.*, **51**, 119 (1952).
- (16) Harned, H. S., and Owen, B. B., "The Physical Chemistry of Electrolytic Solutions," Reinhold Publishing Corporation, New York, 1943, p. 172.
- (17) Gosting, L. J., and Akeley, D. F., *J. Am. Chem. Soc.*, **74**, 2058 (1952).
- (18) Wang, J. H., *J. Phys. Chem.*, **58**, 686 (1954).
- (19) International Critical Tables, Volume 5, McGraw-Hill Book Company, Inc., New York, 1929, p. 63.

Book Notices

A Survey of State Pharmacy Laws. Compiled by JOHN M. SEUS for the National Drug Trade Conference. The American Pharmaceutical Association, Washington, D. C., 1957. vi + 112 pp. 16.5 x 25 cm. Price \$3.

This compilation was prepared primarily to correlate the many different versions of those sections of the various State pharmacy laws pertaining to restriction of the sale of nonprescription drugs, so that the National Drug Trade Conference could use the information in again attempting to prepare a generally satisfactory "model" State pharmacy act. The text includes three general chapters on: Provision of State statutes as to sale of nonprescription drugs; Powers granted to Boards of Pharmacy, Power exercised by Boards of Pharmacy; and Decisions of the courts as to definitions of the term, "patent medicines," "proprietary medicines," "household and domestic remedies," and classification of articles according to restriction of sale, and other related matters. The 63 appendixes include various tables classifying States as to the provisions of their respective laws regulating the sale of drugs by general merchants, and excerpts of specific references to the laws in the 48 States, the District of Columbia, and the Territories of Alaska and Hawaii.

This valuable compilation should be a useful reference for anyone interested in the restrictive sale phase of pharmacy laws. No general index is given but the comprehensive Table of Contents serves the purpose of a detailed guide to the text.

Bacterial Fermentations. CIBA Lectures in Microbial Biochemistry. By H. A. BARKER. John Wiley & Sons, Inc., New York, 1957. vii + 95 pp. 12.5 x 18.5 cm. Price \$3.

This small book presents the three lectures, by the author, on those aspects of bacterial fermentation which he has investigated or which have been of particular interest to him. The subjects covered are: Biological formation of methane, The chemistry of butyric acid-butanol fermentations, and Fermentations of nitrogenous compounds. The main emphasis in each lecture is placed upon the chemistry of the energy-yielding processes. References are given after each chapter and index is appended.

Dermatologic Formulary. From the New York Skin and Cancer Unit, Service of Dermatology. 2nd ed. By FRANCES PASCHER. Paul B. Hoeber, Inc., Medical Book Department of Harper & Bros., New York, 1957. xii + 172 pp. 12.5 x 19 cm. Price \$4.

This book represents a hospital formulary committee's compilation of "The most tried and useful dermatologic prescriptions, together with the briefest and simplest explanations of their uses, indications, and contraindications." The sections of the text are introduced with brief discussions relating to: Topical remedies, Systemic therapy, Articles for clinic use, and Therapeutic aids. A sufficient num-

ber of medicaments are included to circumvent allergic and idiosyncratic reactions and to allow for different patient responses to particular drugs. The abbreviated monographs include descriptions and constituents of drugs and dosage forms.

The book is intended for the dermatologist and does not include procedures for the preparation of dosage forms. An index, with references to prescription numbers and not page numbers, is appended. The book will undoubtedly serve as a model for formularies for other hospital dermatology clinics.

Dictionary of Scientific Terms. By I. F. HENDERSON and W. D. HENDERSON. 6th ed. Revised and enlarged by JOHN H. KENNETH. D. Van Nostrand Co., Inc., New York, 1957. xvi + 532 pp. 14 x 22.5 cm. Price \$12.50.

This dictionary aims to give the pronunciation, derivation, and definition of some 14,000 terms in biology, botany, zoology, anatomy, cytology, genetics, embryology, and physiology. Specific, generic, ordinal, and other systematic names of plants and animals were omitted to limit the size of the book. The method of spelling is in the main that used in Britain with American orthography indicated in cross-references or by reproducing in the original lettering terms culled from U. S. literature. The book should be very useful in the indicated fields of biological sciences.

Handbook of Chemical Data. Edited by F. W. ATACK. Reinhold Publishing Corp., New York, 1957. 629 pp. 10 x 15 cm. Price \$6.75.

This pocket-size book includes tabulations of data that are most frequently consulted by chemists. Its main feature is the small size, which is achieved by judicious omission of less frequently required information which can be found in larger volumes.

Pharmaceutical Calculations. 3rd ed. By WILLIS T. BRADLEY, CARROLL B. GUSTAFSON, and MITCHELL J. STOKLOSA. Lea & Febiger, Philadelphia, 1957. 13 x 20 cm. 325 pp. Price \$4.50.

This revision of a book that has been used by many teachers has been brought up to date and conforms with the usage of U. S. P. XV. It is designed to prepare the student for the use of arithmetic in applied pharmacy. This is done with the aid of many examples and problems. The book is well suited for the modern pharmacy curriculum, and should be useful to practitioners as well as students and teachers.

Organic Syntheses. Vol. 37. Edited by JAMES CASON. John Wiley & Sons, Inc., New York, 1957. vii + 109 pp. 15 x 23 cm. Price \$4.

This volume presents preparative methods for twenty-nine compounds frequently required by organic chemists.

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Determination of Vitamin D by a Chemical Method Involving Chromatography and Color Inhibition*

By J. B. WILKIE, S. W. JONES, and O. L. KLINE

A method for the determination of vitamin D applicable to pharmaceutical products is described in detail. The method involves saponification, extraction, chromatography, and color measurement by a modified antimony trichloride test. An important feature is the use of acetic anhydride which inhibits completely and selectively the vitamin D-SbCl₃ reaction, providing a reaction blank that greatly improves the specificity of the reaction. Of particular concern are the purity of color reagent, and the adsorption index of the magnesium oxide and alumina adsorbents used in the chromatography. Data are presented showing stability of vitamin D in the chromatography, the effect of acetic anhydride in the color reaction, and the validity of corrections based upon the acetic anhydride blank, and upon absorption differences at 500 and 550 mμ wavelengths. The character of the vitamin A degradation interferences is discussed and analytical results of the method applied to a series of pharmaceutical products are compared with bioassay values.

NUMEROUS ATTEMPTS to prescribe a means of estimating Vitamin D, either colorimetrically or spectrophotometrically, have, at best, been only partially successful, and the official methods for this vitamin continue to employ biological procedures. Several years' effort in this laboratory have resulted in refinements of previously described approaches that now permit application of the colorimetric method to pharmaceutical products of low potency. It is possible to carry out determinations with samples containing less than 1,000 U. S. P. units of vitamin D.

The color reactions of both vitamin D₂ and D₃ with SbCl₃ have been the basis for a number

of studies that are comprehensively reviewed by Ewing, *et al.* (1). Among these, the work of Nield, *et al.* (2), showed improved sensitivity and reproducibility of the reagent with the use of acetyl chloride with chloroform. In later work from this laboratory, DeWitt (3) found that ethylene dichloride had a stabilizing effect when substituted for the chloroform solvent.

A different color reaction was described by Sobel, Mayor, and Kramer (4) who used glycerol 1,3-dichlorhydrin with acetyl chloride, and by Campbell (5) who further studied this reaction with vitamin D, improving sensitivity by selection of a suitable wavelength for color measurement.

Grecne (6), in dealing with many phases of the vitamin D determination, developed an iodine trichloride reaction, but found it less sensitive and less specific than SbCl₃.

Several attempts to apply paper chromatography have been made (7-9). Experience with it in our laboratory indicated that separation was

* Received April 14, 1958 from the Division of Nutrition, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D C

We are pleased to acknowledge the interest and help of a number of our colleagues who contributed in many ways to the progress of these studies. We particularly thank Mr. Glen Shue, Mr. Walter Morris, and Dr. Leo Friedman for their assistance.

We also acknowledge, with thanks to Dr. James Waddell of the Dupont Company, Dr. Norris Embree, Distillation Products, Inc., Dr. Saul Rubin, Hoffmann-La Roche, and Dr. Irwin Oleott of Dawe's Laboratories, the contribution of vitamin A or vitamin D preparations of specified purity that were invaluable in the development of these studies.

not sufficiently quantitative to serve as a basis for an analytical method.

Attempts to make use of direct spectrophotometric measurement of vitamin D even after chromatographic purification (3, 6, 10) have been applicable only to high potency materials. Ewing, *et al.* (11), in a later report, state that "for samples containing less than about 4,000 U. S. P. units of vitamin D per Gm. of oil the error begins to rise rapidly, and this value may be taken as a lower limit for the satisfactory determination of vitamin D in oil samples."

Thus the problem of applicability to pharmaceutical products resolves itself primarily to quantitative separation of vitamin D from interfering substances, principally vitamin A and its degradation products. Adsorption column chromatography of the nonsaponifiable fraction has been most useful for such separations. Ewing, *et al.* (11), employed a dual column procedure with superfiltrol separation followed by alumina. These workers observed that the interferences not removed by this chromatography were of two kinds. The first was a residue in the oil, and the second the decomposition product of vitamin A formed in the superfiltrol column.

Refinements in saponification, chromatography, and colorimetry have been the subject of extensive studies in this laboratory, and now provide a basis for workable procedures. The method described here allows a more quantitative separation of the vitamin A degradation products than previous methods. This separation problem cannot be avoided in providing a method useful in control programs since, in most pharmaceutical products, vitamin D is accompanied by vitamin A. Furthermore, vitamin A is useful as a guide in the chromatography. It has not been possible during chromatography entirely to prevent or eliminate vitamin A degradation products that have both chromatographic and spectral characteristics closely similar to those of vitamin D.

Specificity of the colorimetric step was greatly improved when it was observed that acetic anhydride quantitatively inhibits the color reaction of SbCl_3 with vitamin D measured at 500 $m\mu$, but not the reaction with the major interfering substances which are principally vitamin A decomposition products.

PRINCIPLE OF METHOD

The method requires a sample containing approximately 1,000 to 2,000 U.S.P. units (25 to 50 mcg.) of vitamin D for the best precision, but one-half

this amount can be determined by concentration of the final eluate fraction.

Saponification is carried out in alcoholic-KOH with 20 minutes' refluxing.

The nonsaponifiable fraction in petroleum ether is chromatographed, first on MgO that has been standardized with respect to its adsorptive power, for separation of vitamin D from vitamin A and extraneous substances. In a second chromatographing on alumina, vitamin D is separated from some of the fluorescent degradation products of vitamin A present in the sample or formed on the first column.

The vitamin D in the second column eluent is made to react with the acetyl chloride-antimony trichloride reagent to form a color, measured at 500 $m\mu$ by a procedure involving use of an internal standard. Acetic anhydride completely inhibits this reaction, providing a reaction blank, and an additional minor correction of the absorbance value depends upon a reading at 550 $m\mu$, to eliminate any effect of decomposed vitamin A that may escape separation in the chromatography. The vitamin D reference solution in ethylene chloride is evaluated in each determination by multiplying the absorbance at 270 $m\mu$ by a factor that has been carefully determined.

METHOD

Reagents

Vitamin D Reference Solution.—Ethylene dichloride solution of crystalline calciferol or vitamin D_3 . Prepare Stock Solution I to contain 25 mg. in each 100 ml. of petroleum ether or 10,000 units per ml. The Reference Solution is prepared by placing 1 ml. of Stock Solution I in a 50-ml. volumetric flask, removing the petroleum ether with a stream of nitrogen, then making to a volume of 50 ml. with ethylene dichloride, to contain 200 units of vitamin D per ml. or 5 mcg. per ml. All reference solutions are stored in the refrigerator.

KOH.—50% by weight, (780 Gm./liter).

Ethylene dichloride.—1,2-dichloroethane.

Cottonseed Oil.—Used as stabilizing agent of high antioxidant value, and must have a value of not less than 0.2 when measured in the following test: to an 18-mm. test tube add 0.2 ml. of 0.1% aqueous $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1 ml. of 0.1% α, α -dipyridyl in absolute ethanol, and 5 ml. absolute ethanol. Shake, and after 5 minutes read absorbance at 520 $m\mu$ against absolute ethanol set at 100. Then add 0.1 ml. of a solution containing the nonsaponifiable fraction of 1 Gm. cottonseed oil per ml. in petroleum ether. After 5 minutes, read the absorbance, and obtain the absorbance value by subtracting the blank reading. Store in a refrigerator with minimum exposure to air.

Adsorbents— MgO .—ScaSorb 43 (Westvaco Co.) heated in a muffle furnace for three hours at 600°.

Alumina Hyflo-Supercel.—(Johns Manville industrial filter aid).

Alumina.—Alcoa grade F-20.

Treated Alumina.—Alcoa grade F-20. Generally the grading or mesh size distribution of this product is satisfactory. However, not more than 50% of the material should pass a 160-mesh sieve and the material passing the 100-mesh sieve but not the

160-mesh sieve should approximate 50%. The remainder should fall between 60- and 100-mesh. The selected alumina is heated in a muffle furnace for three hours at 600°, and after partial cooling, placed in a tightly closed screw-cap glass jar. After it reaches room temperature the material is sieved through an 80-mesh screen, weighed, then placed in a tared, screw-cap glass jar filled to not more than two-thirds capacity. Distilled water is added, dropwise, with frequent shaking of the capped bottle, until the material contains 3% added water. Shaking is continued for at least fifteen minutes so that no lumps remain, and the material is uniform. After standing overnight, tightly capped, the material when tested has an adsorption index within the range 30 to 40. Since a change in moisture content affects the adsorption index of the reagent, the container must be kept tightly closed, except when removing a portion for use.

Test for Adsorption Index of Alumina

Ten centimeters of the adsorbent to be tested is added and tapped into a settled position in a chromatographic tube 6 mm. (inside diameter) x 20 cm. plugged at its tip with glass wool. One milliliter of solution containing 20 mg./liter of the dye in petroleum ether (F & D Yellow No. 4 of about 99% purity) is then added to the top of the column. Sixteen per cent solution of U. S. P. ether in petroleum ether is then added in small portions from an accurately filled 50-ml. graduated cylinder, while five inches of vacuum is applied.

The number of ml. of this eluent to elute all of the dye is the adsorption index for the adsorbent under test. There may be some difficulty in determining the point at which all of the dye has been eluted from the column. This is accomplished by first collecting the eluate in a 50-ml. beaker until all apparent color in the column is eliminated, and finally by collecting 1-ml. fractions in 1-ml. beakers successively until, when viewed against a white background, the collected eluate is colorless. Removal of individual fractions is easily accomplished at 5-in. vacuum by first slipping the micro bell jar over the edge of the base plate. The total number of ml. to attain the colorless eluate is the required adsorption index.

Retentiveness is both a function of the adsorbed water and the condition of the anhydrous alumina itself. The adsorbed water can be removed by cautious heating at 600° for three hours. Weakened anhydrous alumina caused by too much heating or by heating over 600° should be avoided.

Ethyl Ether.—U. S. P. grade, peroxide-free ether is suitable for the extraction of the saponification mixture. For the second column chromatography, ether, redistilled over KOH pellets, is required. To this latter reagent are added pieces of bright copper strip, to inhibit peroxide formation. This reagent is checked frequently (U. S. P. test) and, at the first indication of peroxides, is discarded.

Petroleum Ether.—Must have a spectral transmission at 300 m μ of at least 85%, in a cuvette the light path of which is 1 cm., against a no-cell blank. Also, in the adsorbent activity test the eluent effect of 10 ml. of petroleum ether by itself must cause a movement of the visible color no more than 1 cm. below the surface of the column. To meet these requirements distillation may be necessary.

Eluting Solutions.—*First column:* 1. 0.5% absolute ethyl alcohol in petroleum ether, 2. 0.1% absolute ethyl alcohol in petroleum ether, 3. petroleum ether. *Second column:* 1. ethyl ether (redistilled over KOH), 2. 5% absolute ethyl alcohol in petroleum ether, 3. petroleum ether. *Test for Adsorption Index of Alumina:* 16% U. S. P. ether in petroleum ether.

Adsorbent Preparation W.—A weak adsorbent composed of 1 part MgO and 5 parts Hyflo-Supercel by weight, thoroughly mixed. When tested by the method described below it has an adsorption index of 5 to 10.

Adsorbent Preparation S.—A strong adsorbent composed of 1 part MgO and 1 part Hyflo-Supercel by weight, thoroughly mixed. When tested by the method described below it has an adsorption index of 20 to 35.

Test for Adsorption Index of MgO Mixtures

Pack tightly with a $\frac{3}{16}$ inch tamper, under 20 inches of vacuum, the mixed adsorbent to be tested into a 1.0 to 1.2-cm. tube fitted with a sealed-in fritted disk to a height of 1.5 cm. With the vacuum off, add 1 ml. petroleum ether solution containing 20 mg./liter F&D Yellow No. 4 of about 99% purity. Add about 2 ml. of 10% absolute alcohol in petroleum ether from an accurately filled 50-ml. graduated cylinder. Increase the vacuum to 20 inches and continue to add the 10% eluent until most of the dye band is eluted. Remove and discard the collected eluate. Collect successive 1-ml. fractions in 1-ml. beakers until, when viewed against a white background, the eluate is judged colorless. Removal of individual fractions is easily accomplished at 5-in. of vacuum by slipping the edge of the micro bell jar over the edge of its base plate. The number of ml. of 10% alcohol in petroleum ether to attain the first colorless eluate fraction is the adsorption index of the magnesium oxide mixture.

Antimony Trichloride Reagent.—*Solution A*—Using antimony trichloride, U. S. P., in a dry, crystalline form, packed in a well-sealed glass-stoppered bottle, dissolve approximately 113 Gm. in sufficient ethylene dichloride to make 500 ml. To avoid moisture contamination, this is done quickly by emptying, without weighing, a previously unopened $\frac{1}{4}$ -lb. bottle of antimony trichloride into about 400 ml. ethylene dichloride. Add approximately 2 Gm. anhydrous alumina, mix thoroughly, and filter the solution through filter paper into a clear glass reagent bottle, then make to a previously marked 500-ml. volume with ethylene dichloride. This solution is colorless, or nearly so, and must have a spectral transmittance of at least 85% at 500 m μ , measured against the ethylene dichloride solvent.

Solution B.—In a hood add 100 ml. acetyl chloride to 400 ml. ethylene dichloride with mixing, and store in a glass-stoppered bottle.

Color Reagent.—Mix, at least one-half hour before use, 45 ml. of Solution A and 5 ml. of Solution B, and keep in a glass-stoppered flask tightly stoppered until needed. This solution is satisfactory until it becomes colored. It is best to prepare it fresh on the day of use, although it may be useful over a period of a week.

Color Inhibitor.—A solution containing equal volumes of acetic anhydride and ethylene dichloride.

APPARATUS

Chromatographic Tubes

Tube for First Column Chromatography.—2.5 cm. in diameter x 20 to 30 cm. in length, with coarse fritted disk, and sealed to an 8 mm. x 5 cm. exit tube.

Tube for Second Column Chromatography.—6 mm. (inside diameter) x 20 cm. in length, with the lower 5 cm. pulled out to form a tapered constricted exit. About 1 cm. of the upper part of the constricted section is plugged with glass wool.

Tube for MgO Adsorption Index Test.—1 cm. in diameter by 10 cm. in length, with medium fritted disk, and sealed to an 8 mm. x 5 cm. exit tube.

Supplementary Evaporation and Chromatographic Apparatus

A vacuum micro bell jar large enough to hold a 100-ml. beaker is used in applying the vacuum and collecting the eluates. The vacuum either from the line or from a water vacuum aspirator is controlled by a vacuum gauge and a screw clamp bleeder. A stopcock or screw clamp bleeder on a T tube may be used to control the vacuum delivery to the gauge and chromatography assembly. Fifty- or 100-ml. beakers, a water vacuum aspirator attached to a vacuum gauge, and a three-way stopcock device with interchangeable Florence flasks having standard glass joints may be used to expedite change of solvents (12). The use of manual automatic pipets is important because of the obnoxious or potentially harmful nature of the reagents and solvents used.

Ultraviolet Lamp.—A lamp source of weak ultraviolet is required for observing the fluorescent bands on the chromatographic column. The lamp should provide radiation in the longer (300 m μ) region. Suitable lamps may be constructed or are commercially available. For commercial lamps a narrow aperture or screen may be necessary to reduce the amount of destructive radiation.

Standardization of Test Tubes for Photometric Measurement

Photometrically matched tubes are required. A solution of F&D Orange No. 2 is particularly useful for this purpose.

Photometer.—Any direct reading photometer or monochromator which has a sufficiently narrow absorption band at 500 and 550 m μ to provide linearity is suitable.

PROCEDURE

Sample

A desirable size of sample is one that contains 1,000 to 2,000 units of vitamin D. A smaller amount can be determined by reducing the volume of the final solution used for color measurement. However, the concentration of the final solution should not fall below 50 units per ml. A condition to be observed also is the amount of vitamin A contained in the sample. If the sample contains little or no vitamin A, approximately 3,000 units are added to provide the pilot bands necessary in the chromatography. However, the total amount present must not exceed the capacity of the first adsorption column which is usually in the range of 20,000 to 25,000 U. S. P. units of vitamin A.

Capsules or tablets are disintegrated by adding 10 ml. H₂O, warming on a steam bath with the 200-ml. Erlenmeyer flask at an angle, for ten minutes. It may be necessary to crush them with a flattened glass rod, and to warm for an additional five minutes. A metal clamp on the neck of the flask is useful for holding the flask at an angle, and for holding it immersed in the steam bath during the subsequent saponification step.

Saponification

To a measured amount of sample in a reflux flask add 1 ml. cottonseed oil, and 2.5 ml. of 50% KOH for each Gm. of sample taken, but not less than 15 ml. Then add 50 ml. of 95% ethanol for samples of less than 5 Gm. For samples of between 5 and 10 Gm. of fat or oil, add 75 ml. of 95% ethanol. Boil vigorously twenty minutes (thirty minutes for 5 to 10-Gm. samples of fat or oil) immersed in a steam bath, with a suitable reflux condenser. Cool the saponification container. Transfer contents to a 500-ml. separatory funnel, rinsing with 50 to 70 ml. of distilled water in several portions, adding each portion to the separatory funnel. Then add 50 to 100 ml. of ethyl ether. Good separation and relative freedom from emulsions at this step are obtained if the volumes of water, alcohol, and ether are maintained in approximately a 1:1:1 ratio. Shake vigorously and allow to stand about two minutes.

Separate the aqueous layer into another 500-ml. separatory funnel, and extract it three more times with 30-ml. portions of ether, adding each ether layer in turn to the original ether extract. In the case of slow separation or emulsion formation, add 2 to 5 ml. of 95% alcohol and swirl gently. Solid material in the aqueous phase is forced through the funnel with a wire of suitable size.

Pour 100 ml. of distilled water through the combined ether extracts, wait five or more minutes for complete separation, and discharge the lower water layer into another separatory funnel. For 10-Gm. oil samples, 200 ml. of water may be necessary here. This aqueous fraction is then extracted twice with 50-ml. portions of ether and the resulting layers added to the original ether extract. These two ether extractions should be accomplished with vigorous shaking. Good separations will generally require subsequent gentle swirling with additions of about 5 ml. of ethanol. Allow two to five minutes for separations in this step. Pour two 100-ml. portions of distilled water through the combined ether extracts without shaking, allow to separate, and discard these aqueous fractions. Add about 10 ml. of water and shake vigorously. If there is a slight persistent emulsion formed at the interface, dilute by pouring water through the ether extract, and discard the aqueous layer.

Where particularly persistent emulsion occurs, or where precipitates gather at the interface, gather as much as possible of this material at the interface by shaking with about 3-ml. volumes of water or dilute alkali solution. Isolate the interface material in a separatory funnel set aside for this purpose. It is important to collect in one funnel all of the separations of such interface material even though some of the solvent portions are unavoidable. This interface fraction is then shaken with about 25 ml. of petroleum ether which will likely become cloudy.

The cloudiness is removed by swirling with about 3 ml. of 95% alcohol. The clear petroleum ether solution is then added to the original solvent extract, and in like manner, the interface fraction is extracted two more times with 25 ml. of petroleum ether and 3 ml. of 95% alcohol, adding each to the original ether extract.

Add successive portions of distilled water to the ether extract with gentle agitation, removing each until the rinse water is free of alkali as shown by a phenolphthalein test. Drain off the last small fraction of water and discharge ether extract into a 300-ml. tall beaker containing about 5 Gm. anhydrous Na_2SO_4 . Stir for about two minutes, and transfer the ether extract with ether rinsing, to another 300-ml. tall beaker. Evaporate the extract to about 30 ml. on a steam bath and transfer to the flask of the evaporation apparatus for final evaporation under vacuum.

The evaporation flask is placed in the steam bath until a volume of 5 ml. remains. The flask is then placed under vacuum in a water bath at a temperature below 40° until evaporation is complete, as indicated by appearance and an increase in vacuum. Introduce petroleum ether, dissolve the residue, and make to a volume of 10 ml. This is the sample extract.

First Column Preparation.—Using a rod approximately $\frac{1}{4}$ inch in diameter, tamp W adsorbent tightly into the large chromatographic tube, under 25 inches of vacuum, to form a column segment 2.5 cm. in height; make the surface level and smooth.

Under the same vacuum add S adsorbent to a height of approximately 10.5 cm. With the surface level, press the material firmly with a cork attached to a glass rod so that this section is about 8 cm. in height. The cork should have approximately the same diameter as the tube, fitting loosely, to provide even pressure throughout the cross section of the tube.

Complete the column by the addition of a layer of about 1 cm. of anhydrous Na_2SO_4 .

First Column Chromatography—Sample Addition.—With the column under 5 inches of vacuum, pour 30 ml. of petroleum ether on the column, slowly down the side of the slightly tipped tube. When 20 ml. of petroleum ether remain above the Na_2SO_4 layer, release the vacuum and add the sample extract, again pouring carefully down the side of the slightly tipped tube. Apply 20 inches of vacuum and, as the surface of the liquid disappears into the Na_2SO_4 layer, reduce the vacuum to 5 inches and quickly add 3 small portions (2 to 5 ml.) of petroleum ether down the side of the tube, allowing each portion to move into the Na_2SO_4 layer before adding the next. Then carefully add 25 ml. petroleum ether. As the surface of this addition reaches the Na_2SO_4 layer, add the eluting solution containing 0.1% alcohol in petroleum ether in 10-ml. portions. In the period following the first addition of this eluent, the fluorescent vitamin A begins to move in a compact band, and separating ahead of it is a narrow yellow fluorescent band. The movement of these bands can be followed by frequent brief inspections with the ultraviolet lamp. At the end of fifteen minutes increase the vacuum to 20 inches; and by the end of 30 minutes, the fluorescent bands should move one-quarter to one-half the length of the upper segment of the column.

If the lower band has moved one-half the length of the upper segment in less than fifteen minutes, its movement is retarded by substituting petroleum ether for the 0.1% alcohol eluent, and by keeping the vacuum at 5 inches throughout the elution. (With some experience it is possible to make use of a chromatogram that develops rapidly whenever there is a 2 to 3-cm. separation between the bands. In such case, increase the vacuum to 20 inches, change the collection flask when the yellow fluorescent band reaches the junction, and change to petroleum ether for eluting when the vitamin A band is between 1 and 2 cm. from the junction.)

If the bands have failed to move as described above with the vacuum at 5 inches, add 5 ml. of the 0.5% alcohol in petroleum ether eluent, followed by 25 ml. of the 0.1% eluent, repeating this sequence of additions if necessary, until movement is apparent, and the band has moved one-half the length of the upper segment. Then increase the vacuum to 20 inches.

At this point the separation of the two fluorescent bands should be greater than 1 cm. and when the lower yellow fluorescent band reaches the junction of the two adsorbents, the eluate is discarded and a clean collection beaker is placed in the bell jar.

Elution is continued by the addition of the 0.1% alcohol eluent until the vitamin A band reaches a point 1 cm. above the junction, then petroleum ether alone is used as the eluent until the junction is reached.

When the vitamin A band reaches the junction, the vacuum is reduced to 5 inches, and elution is continued with petroleum ether until the lowest tip of the vitamin A band touches the fritted disk. At this point collection is discontinued, using great care to collect as much eluate as possible without including any vitamin A.

The stem of the chromatographic tube is rinsed with petroleum ether into the collection beaker, and the eluate is transferred to the evaporation apparatus.

The evaporation flask is placed in the steam bath and evaporation continued until a volume of about 5 ml. remains. The flask is then placed in a water bath at a temperature below 40° , under vacuum, until evaporation is complete, as indicated by appearance and an increase in vacuum. Introduce petroleum ether, dissolve the residue, and make to a volume of 5 to 10 ml. This eluate solution is used for the second column chromatography.

Second Column Preparation.—Add to the small chromatographic tube 20 cm. of treated alumina, avoiding extending the column into the enlarged section of the tube. The column is made sufficiently compact by tapping the side of the tube during and after the addition of the adsorbent.

Second Column Chromatography.—With the column under 20 inches of vacuum, and a 25-ml. collecting beaker in place, add the eluate solution from the first column, rinsing the flask with a small amount of petroleum ether. As soon as the eluate solution has moved into the column, reduce the vacuum to 5 inches, and add 2.0 ml. of reagent ethyl ether. When this has moved into the column add 7 ml. of petroleum ether and allow it to move into the column. Release the vacuum and remove the eluate, identifying it as fraction No. 1. With a clean 10-ml. collection beaker continue elution,

under 5 inches of vacuum, with the addition of 20 ml. of 0.1% alcohol eluent. Three separate eluate fractions are collected, each approximately 5 ml. and identified as fractions No. 2, No. 3, and No. 4. These fractions are precautionary to make certain that the chromatography is effective. Normally, when these fractions are exposed to the weak ultraviolet light, fraction 1 fluoresces strongly, fraction 2 may show some, and fractions 3 and 4 are without fluorescence. If fraction 3 is nonfluorescent, and fraction 4 shows fluorescence, then the latter is added to fraction 5 which is fluorescent. But if all fractions show fluorescence, the chromatography has been inadequate, and should be repeated. For collection of eluate fraction No. 5 in a 50-ml. collection beaker, 25 ml. of 5% alcohol eluent is added with the vacuum at 20 inches.

Evaporate the eluate fraction No. 5 in a 50-ml. flask in the evaporation apparatus. The evaporation flask is placed in the steam bath until a volume of 5 ml. remains. The flask is then placed in a water bath at a temperature below 40°, under vacuum, until evaporation is complete, as indicated by appearance and an increase in vacuum. Alternately, the eluate fraction may be evaporated on a steam bath to a volume of about 2 ml. The beaker is then removed from the steam bath and evaporation is completed under a light stream of nitrogen. Introduce 5 ml. of ethylene dichloride, dissolve residue, and transfer to a 10-ml. volumetric flask, rinsing with several small portions of ethylene dichloride. Make to a final volume of 10 ml. This is the sample solution to be used in the color reaction.

Color Reaction.—All colorimetric measurements are made in the same tube or in matched tubes. The tubes must be rinsed with ethylene dichloride three times after each use. Exactly thirty seconds after the addition of the color reagent, the transmittance reading of 500 $m\mu$ is taken with the instrument set at 100% transmittance with ethylene dichloride. A second reading is made at 550 $m\mu$ within ninety seconds after the color reagent addition. An internal standard is used to compensate for possible sample effects on color development. The color reagent is added rapidly. For this, a device such as a 10-ml. graduated cylinder cut off at the 7-ml. mark is practical, or an automatic measuring pipet may be used. The three color reactions are carried out in the following order: (a) 1 ml. sample solution + 1 ml. reference solution + 5 ml. color reagent. (b) 1 ml. sample solution + 1 ml. ethylene dichloride + 5 ml. color reagent. (c) 1 ml. sample solution + 1 ml. color inhibitor + 5 ml. color reagent.

Standardization of Reference Solution.—The vitamin D reference solution in ethylene chloride is evaluated in each determination by measuring its absorbance at 270 $m\mu$. Concentration of vitamin D, in units per ml., is calculated:

$$A_{(r) 270} \times 860 = \text{units per ml.}$$

Calculation.—Convert transmittance readings to the corresponding absorbance values, then with:

$$A_{(s+r) 500} = 500 m\mu \text{ absorbance of sample solution} \\ + \text{reference solution} \\ A_{(s) 500} = 500 m\mu \text{ absorbance of sample solution}$$

$$A_{(s+r) 500} = 500 m\mu \text{ absorbance of sample solution} \\ + \text{color inhibitor} \\ A_{(s) 550} = 550 m\mu \text{ absorbance of sample solution} \\ A_{(s+r) 550} = 550 m\mu \text{ absorbance of sample solution} \\ + \text{color inhibitor}$$

$$A_{REF} = A_{(s+r) 500} - A_{(s) 500}$$

$$\text{Reference concn. (units/ml.)} =$$

$$\frac{A_{REF}}{K \text{ (Concn. of vitamin D per unit of absorbance)}}$$

$$\left[\frac{A_{(s) 500} - A_{(s+r) 500}}{A_{(corr. S) 500}} - 0.67 (A_{(s) 550} - A_{(s+r) 550}) \right] = \\ A_{(corr. S) 500} \times K = \text{vitamin D content of sample solution (units/ml.)}$$

Units per ml. sample solution/ C = vitamin D content of sample, where C is the concn. in Gm., capsules, or tablets per ml. of colorimetrically evaluated sample solution of the material taken for analysis.

DISCUSSION

Preparation of Sample.—Although oils are readily saponified there is advantage in treating capsule or tablet samples with a small amount of hot water to speed disintegration and to reduce the time necessary for complete saponification. With such treatment of oil-insoluble coatings the twenty-minute saponification time is generally adequate.

Vitamin D has been shown to be unstable on the MgO chromatographic column, in the absence of antioxidant and samples of unknown composition that do not contain antioxidant have been encountered. The addition of 1 ml. fresh cottonseed oil to each sample assures stability of the vitamin throughout the procedure. Results of comparison of vitamin D chromatographed with and without addition of cottonseed oil are shown in Table I.

TABLE I.—RECOVERY OF VITAMIN D FROM CHROMATOGRAPHIC COLUMN IN PRESENCE AND ABSENCE OF ANTIOXIDANT

Without Cottonseed Oil			With Cottonseed Oil ^a		
Vitamin D Added, Units/ml.	Vitamin D Recovered, Units/ml.	Recovery, %	Vitamin D Added, Units/ml.	Vitamin D Recovered, Units/ml.	Recovery, %
150	129	86	200	192	96
200	176	88	200	195	98
200	114	57	200	192	96
200	128	64	200	191	96
			200	193	97

^a 1 ml. added to each sample.

Saponification.—Immersion of the saponification flask in a steam bath is prescribed. Incomplete saponification leads to difficulties in control of the chromatography. An efficient reflux condenser over a flask heated on a hot plate may cool and slow the process to such an extent that saponification is not completed in the twenty-minute period. In such a case the presence of a small amount of fat in the solution for chromatography causes rapid elution of the visible bands and prevents the necessary separation of vitamins A and D.

Extraction.—The procedure described is essentially that used for fish-liver oils in determining vitamin A content by the A. O. A. C. method. The additional treatment required for samples in which resistant emulsions or precipitates at the solvent interface occur has been found quite satisfactory in practice. However, relatively few samples that require such treatment have been encountered.

Chromatography.—The chromatographic procedure is the foundation of this method and the culmination of long experience and many experiments. Important improvement resulted from the knowledge that sufficient uniformity may be assured by tempering the MgO with heat and Hyflosupereel, and the alumina adsorbent with heat and water. Adsorptive capacity of the alumina is maintained within a narrow and workable range by heating it at 600° for three hours, then adjusting the moisture content to 3%. Assurance of suitability of the adsorbents used by determining adsorption index is essential. Also, particle size of the alumina is important.

Most pharmaceutical samples containing vitamin D also contain vitamin A. Since the method described here depends upon the vitamin A and yellow fluorescent pilot bands, a measured amount of vitamin A must be added to those samples devoid of it. Separation of all vitamin A is accomplished on the first column. To assure this separation it is important that a good division is obtained between the vitamin A band and the yellow fluorescent band, that moves ahead of it. In this case, the vitamin D moves well ahead of the vitamin A, but is too close to the yellow fluorescent band to permit adequate separation of these two on this column. The fraction eluted ahead of the yellow band eliminates unidentified interference, probably sterol in nature. Great care and some skill are necessary in carrying out this chromatography successfully. There is evidence that the yellow fluorescent band material contains both colored and colorless fluorescent substances. Further evidence indicates that these substances arise largely as degradation products of vitamin A. For example, with repeated chromatography of the vitamin A band on the MgO column, additional yellow fluorescent band material appears in approximately the same proportion in each separation preceding the vitamin A band. We have observed this as well when starting with vitamin A of high purity. It is usually not difficult to separate the yellow material from vitamin D in the alumina column. It has not been possible, however, completely to eliminate a colorless fluorescent substance from the vitamin D fraction, a substance which has spectral absorption in the ultra-violet closely similar to that of vitamin D, but which causes little interference in the SbCl₃ color reaction. Because of an inability to accomplish this complete separation, determination of absorbance of the vitamin D eluate fraction at 270 mμ is not a reliable measure of vitamin D.

In Fig. 1 are shown absorption curves for vitamin D₃ in ethylene chloride (Curve A) and in ethyl alcohol (Curve B). The yellow fluorescent fraction that is separated from the vitamin D fraction in the second column has the absorption characteristics shown in Curves C and D. The vitamin D eluate fraction, Curve E, shows characteristic absorption in the 270 mμ region, but is displaced to the right at the higher wavelengths. The absorption of

vitamin A epoxide, as described by Karrer (13), is represented by Curve F. That vitamin A epoxide, or a degradation product of vitamin A having closely similar absorption characteristics, is the contaminant of the vitamin D eluate fraction from the second column chromatography is demonstrated in Fig. 2. A sample of vitamin A, free of vitamin D, was taken through the chromatography, and absorption of the fraction that would be expected to contain the vitamin D was measured. This is shown as Curve C. Again Karrer's vitamin A epoxide is included as Curve B, and for reference Curve A is the absorption of vitamin D in ethylene chloride. It is quite clear that the chromatography used does not provide a separation sufficiently complete to rely upon direct spectrophotometric absorption of the final eluate as an estimate of the vitamin D present.

The procedure for the SbCl₃ vitamin D color reaction is essentially DeWitt's modification (3) of Nield's reaction. No other color reaction with vitamin D has been found to equal the sensitivity of that with the SbCl₃ acetyl chloride reagent. It is quite susceptible to interference, however, where vitamin A or its degradation products are present. We have learned that in the presence of acetic anhydride the color reaction with vitamin D is completely inhibited, while that with the common interfering substance is inhibited to a small extent or not at all.

The effect upon vitamin D alone is shown in Table II.

TABLE II.—EFFECT OF ACETIC ANHYDRIDE UPON THE VITAMIN D₃-SbCl₃ REACTION

Units, Vitamin D/ml.	Absorbance at 500 mμ Without Acetic Anhydride	With Acetic Anhydride
100	0.120	0.001
200	0.237	0.000
400	0.488	0.001

TABLE III.—APPLICATION OF ACETIC ANHYDRIDE INHIBITION TO SPECTROPHOTOMETRIC EVALUATION OF VITAMIN D-SbCl₃ COLOR

Sample	1. Total Absorbance at 500 mμ	2. Absorbance with Acetic Anhydride	3. Absorbance Correction from 550 mμ Reading	4. Vitamin D Absorbance
1	0.272	0.076	0.015	0.181
2	0.196	0.043	0.005	0.148
3	0.161	0.051	0.017	0.093
4	0.268	0.078	0.018	0.172

In Table III are given examples of absorbance values from Beckman Spectrophotometer Model B readings of sample solutions prepared from four different samples. In the description of the method these readings correspond as follows:

Column 1 is $A_{(s)} 500$; column 2 is $A_{(s+i) 500}$; column 3 is $0.67(A_{(s) 550} - A_{(s+i) 550})$; column 4 is column 1 minus the sum of columns 2 and 3.

It is clear that the total absorbance values shown in column 1 do not accurately represent the vitamin D present. The interference absorbance shown in column 2 has been an important source of error in earlier work. The correction shown in column

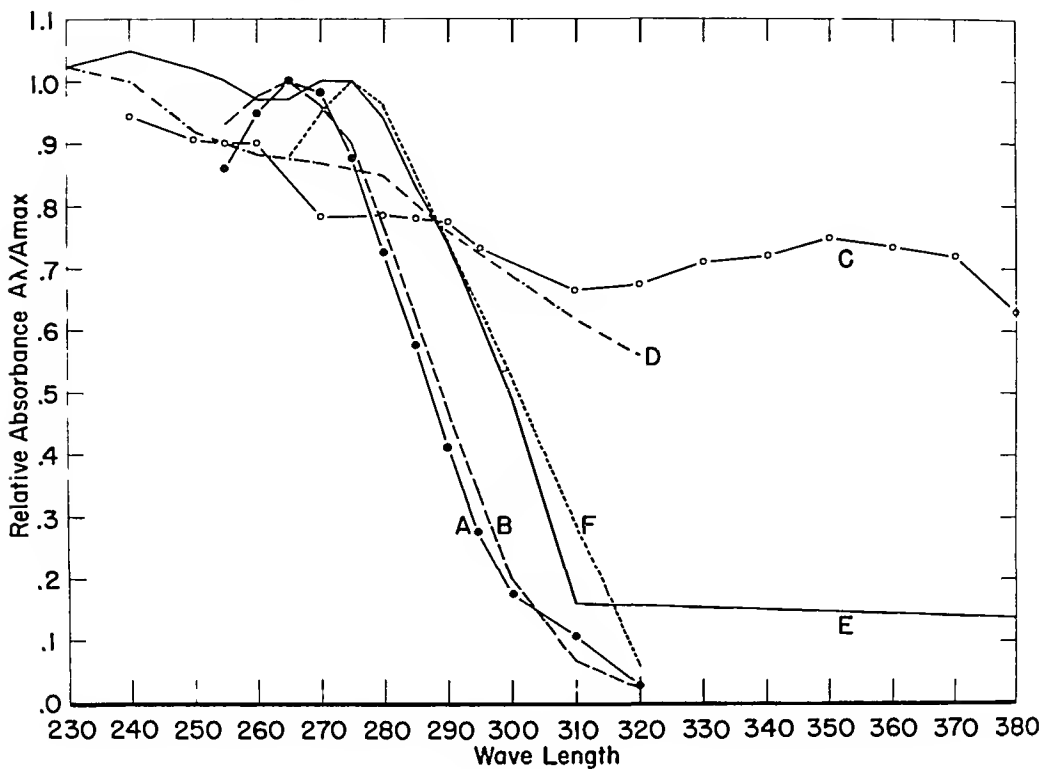


Figure 1.

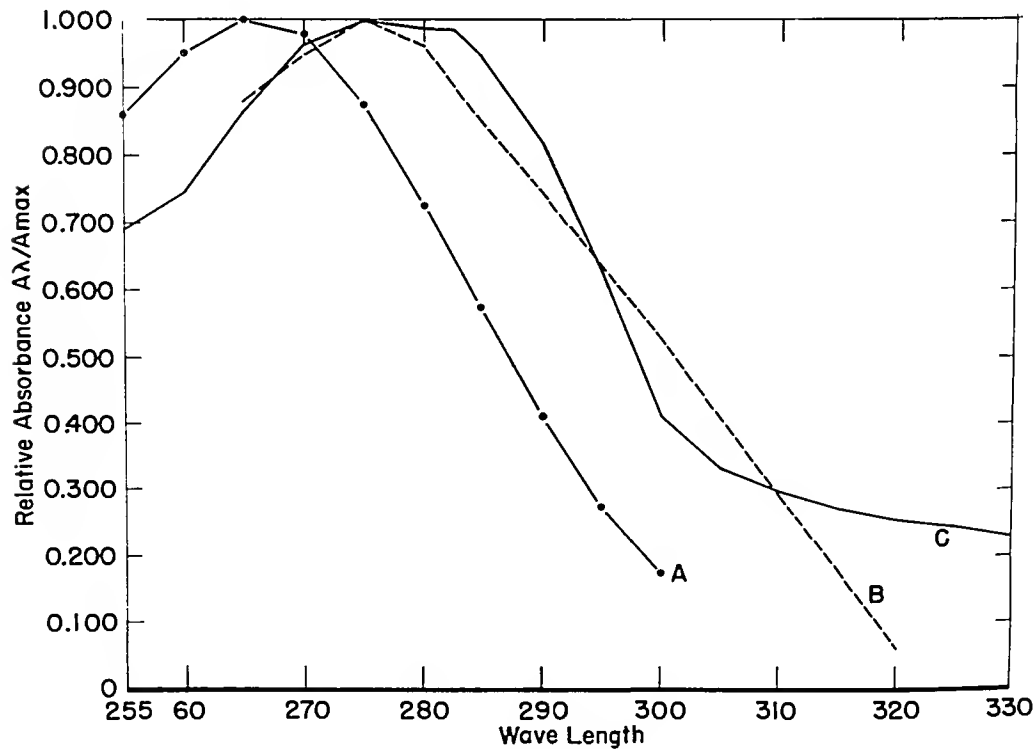


Figure 2.

3 is small and frequently negligible with the chromatography used.

Basis for the small correction indicated in column 3 is given in Table IV, the data for which were obtained as follows

The method was applied to four samples that contained vitamin A, but no vitamin D. In the color test of the final eluate, the so-called vitamin D eluate fraction from the second column, the absorbance values for the sample (Tube $A_{(3)}$), and the sample plus acetic anhydride (Tube $A_{(3+1)}$) were determined at 500 and at 550 $m\mu$

The difference between tubes $A_{(3)}$ and $A_{(3+1)}$ at each wavelength are expressed as Δ absorbance, shown in Table IV. The ratio of the differences is 0.67, which is in close agreement with a large number of such determinations. Since the vitamin D- $SbCl_3$ color has no absorption at 550 $m\mu$, it is possible to correct for non-vitamin D color by subtracting from the total absorbance, two-thirds of the difference observed at 550 $m\mu$. This is the correction shown in column 3 of Table III. Nield (2) and Greene (6) attempted a correction based upon the 550 $m\mu$ absorption. This non-vitamin D absorption seldom exceeds 10% of that of vitamin D. When the correction is excessive a rechromatographing of the final eluate is desirable.

In color measurements of solutions containing from 50 to 1,000 units of vitamin D per ml of final solution, Beer's law was found to apply. The smallest deviations were observed between 100 and 400 units per ml., and it is recommended that final concentration be kept within these limits

TABLE IV.— Δ ABSORBANCE VALUES AT TWO WAVELENGTHS FOR THE VITAMIN D ELUATE FRACTION

Sample	Δ Absorbance at 500 $m\mu$	Δ Absorbance at 550 $m\mu$	A_{500}/A_{550}
1	0.019	0.029	0.66
2	0.025	0.036	0.70
3	0.018	0.025	0.72
4	0.021	0.035	0.60
		Average	0.67

The $SbCl_3$ reagent, Solution A, has been found to cause difficulty if its transmittance of 500 $m\mu$ falls below 85%. An occasional bottle of the solid $SbCl_3$ is found to contain color which, in solution, absorbs in the critical region. Also, moisture present in Solution A may cause a cloudiness that alters transmittance. Solution A is usually stable after the treatment with anhydrous alumina, but should be checked spectrophotometrically at frequent intervals. Any indication of change requires additional treatment with the anhydrous alumina. If color is excessive, it is necessary to prepare fresh reagent. Color change is readily noticed when the solution is stored in a clear glass bottle.

Since the color reaction is affected by substances other than vitamin D contained in the final sample solution, the use of an internal standard is essential. Variation between aliquots and from sample to sample, in the relation of absorbance to vitamin D, is shown in Table V. The values shown are the ratio of units of vitamin D per ml. of final solution

to absorbance value. This is the K value obtained in the calculation of results.

TABLE V.—VARIATION IN K RATIOS OF VITAMIN D TO ABSORBANCE VALUE IN THE COLOR TEST

For Aliquots of Final Solution of a Single Sample	For Final Solutions from Separate Chromatographings of a Single Sample	For Final Solutions of Different Samples
1140	880	989
1070	1022	985
1150	1065	824
1050		1015
Coeff. of var.	5.2	14.3
4.45		

It is apparent that there is a greater variation of K between samples than between aliquots of the same sample. Error from these variations is controlled by the use of the internal standard.

TABLE VI.—VITAMIN D CONTENT OF SEVERAL PHARMACEUTICAL PREPARATIONS

Sample	Label Declaration (U S P Units)	Analysts' Results (U S P Units)		Bioassay Results (U S P Units)
		Analyst A	Analyst B	
1 Elixir	1,300 units/5 ml	1,450	1,450	1,950
2 Syrup	533 units/ml	605	600	675
3 Liquid	2,000 units/5 ml	1,345	1,315	1,500
4 Tablet	2,500 units/tab	3,080	2,910	3,200
5 Capsule	1,000 units/cap	1,430	1,005	1,140
6 Capsule	2,500 units/cap	2,740	3,070	3,150
7 Yeast		78,875		75,000
8 Syrup	1,000 units/0.6 ml	1,100	1,050	1,280
9 Capsule	1,200 units/cap	1,320	1,150	1,140

APPLICATION

Although the method in its various stages of development has been used in this laboratory over a period of several years, and has been applied to a variety of products, the most effective use has been in the category of pharmaceutical preparations. The list shown in Table VI illustrates the types of material most successfully analyzed. Also from the results of two independent analysts the uniformity of results can be judged. The analytical values are compared with the bioassay result for each sample listed. The bioassays were not conducted specifically as a part of this study, but results were obtained as part of the routine assay program of the laboratory. It is clear that the results of the two methods are in the same range.

We have had little experience in applying the method to foods containing vitamin D. Here the problem is to saponify and extract sufficient material to provide approximately 1,000 to 2,000 units of vitamin D. With a suitable extract there would appear to be no great difficulty in extending the method to foods.

REFERENCES

- (1) Ewing, D. T., Kingsley, G. V., Brown, R. A., and Emmett, A. D., *Ind Eng Chem, Anal Ed*, 15, 301 (1943)
- (2) Nield, C. H., Russell, W. C., and Zimmerli, A. J., *Biol Chem*, 136, 73 (1940).
- (3) DeWitt, J. B., *Ind Eng Chem, Anal Ed*, 18, 117 (1946)
- (4) Sobel, A. E., Mayor, A. M., and Kramer, B., *ibid*, 17, 160 (1945)
- (5) Campbell, J. A., *Anal Chem*, 20, 766 (1918)

- (6) Greene, J., *Biochem J*, 49, 36(1951). *ibid*, 49, 45 (1951), 49, 54(1951), 49, 232(1951), 49, 243(1951)
 (7) Kodicek, E., and Ashby, D. R., *Biochem J*, 57, xii (1954), *ibid*, 57, xiii(1954)
 (8) Davis, R. B., McMahon, J. M., and Kalnitsky, G., *J Am Chem Soc*, 74, 4483(1952)
 (9) Kritchevsky, D., and Kirk, M. R., *Arch Biochem Biophys* 35, 346(1952)

- (10) Ewing, D. T., Powell, M. J., Brown, R. A., and Emmett, A. D., *Anal Chem*, 20, 317(1948).
 (11) Ewing, D. T., Schlabach, T. D., and Powell, M. J., *ibid*, 26, 1406(1954)
 (12) Parrish, D. B., *J Assoc Offic Agr. Chemists*, 39, 126 (1956)
 (13) Karrer, P., and Jucker, E., *Helv Chim Acta*, 30, 559 (1947)

An Identity Test for Iodinated Derivatives of Tyrosine and Thyronine, Particularly Triiodothyronine and Thyroxine*

By PAUL Z. ANTHONY and JAMES E. GEEVER

The Kendall-Osterberg color reaction for thyroxine has been applied to other iodinated phenolic amino acids. It provides a ready means of identifying individual members of two families of compounds.

A RAPID METHOD for distinguishing between the various iodinated derivatives of tyrosine and 3,5 diiodothyronine has been developed using the method of Kendall and Osterberg (1). In their work on the chemical identification of thyroxine these investigators noted that thyroxine gives a red color when treated first with nitrous acid and then with ammonia. Ingvaldsen and Cameron (2) found that this color reaction occurs with both diiodotyrosine and thyroxine. They noted that the color is given by a phenolic compound containing two iodine atoms *ortho* to the hydroxy group, they found that the color is not given by dibromotyrosine, nor by a large number of other benzene derivatives. Morton and Chaikoff (3) adapted the reaction to a photometric determination of thyroxine and diiodotyrosine in thyroid tissue. Roche and Michel (4-6) conducted a detailed study of the reaction in the analyses of thyroid gland and iodinated proteins. Adamson, *et al.*, (7), used the reaction for the determination of synthetic sodium L-thyroxine, particularly in tablets containing thyroxine as the active ingredient in microgram quantities. Gross and Pitt-Rivers (8) used 40%

sodium hydroxide instead of concentrated ammonium hydroxide to obtain a brownish-orange color with both thyroxine and 3,5,3'-triiodothyronine; the solution had a maximum absorption between 460 and 470 m μ .

In our laboratory the Kendall-Osterberg reaction gave characteristic colors for iodo-derivatives of tyrosine and 3,5-diiodothyronine.

EXPERIMENTAL AND RESULTS

The optimal concentration of an iodinated compound for color development under the conditions of the test is 0.045 to 0.050 microequivalents per cc. of final solution. The test is run as follows using 3,5,3'-triiodothyronine as an example.

A 20-mg portion of L-triiodothyronine hydrochloride (0.03 milliequivalent) is dissolved in 50 cc of 95% ethanol (wherever an alcohol-insoluble amino acid is used, a few drops of dilute hydrochloric acid are added to bring about solubility). A 2-cc. aliquot is placed in a 25-cc volumetric flask and diluted with 4.5 cc of 95% ethanol and 12.2 cc. of acidic sodium chloride reagent (170 Gm of sodium chloride in sufficient 1 N hydrochloric acid to make 1000 cc). A 2.5-cc portion of freshly prepared 1% sodium nitrite solution is added, and the resulting solution placed in the dark for twenty minutes. A yellow color develops. Addition of approximately 3.8 cc of concentrated ammonium hydroxide (up to the graduated mark) produces an orange color.

This test was also performed on dihydroxyphenylalanine and tryptophan and the results, as well as results on various iodinated phenolic amino acids, are listed in Table I.

Spectrophotometric absorption curves of the colored products of triiodothyronine and thyroxine exhibited absorption maxima at 475 and 495 m μ , respectively (Fig 1). This distinct difference in the maxima showed that the orange color of triiodo-

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The authors are grateful for the assistance and advice of Dr. L. G. Ginger and Dr. N. J. Kartinos of the Research Department of Baxter Laboratories.

thyronine is not a dilution of the pink color of thyroxine but is characteristic of triiodothyronine.

DISCUSSION

Roche and Michel (5) reported that, in addition to thyroxine and diiodotyrosine, the reaction is

TABLE I—KENDALL-OSTERBERG COLOR REACTION WITH AROMATIC AMINO ACIDS

Amino Acid ^a	Color
Tyrosine	Colorless
Monoiodotyrosine	Orange
Diiodotyrosine	Pink
3,5-Diiodothyronine	Colorless
3,5,3'-Triiodothyronine	Orange
Thyroxine	Pink
Dihydroxyphenylalanine	Amber
Tryptophan	Colorless

^a The first 6 compounds were prepared analytically pure in our laboratory. The dihydroxyphenylalanine was Eastman's white label grade. The tryptophan was an analytically pure compound obtained from Schwarz Laboratories, Inc.

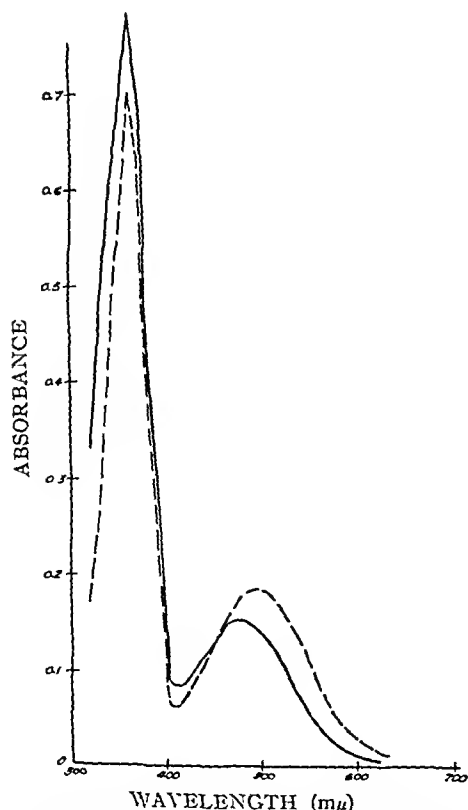


Fig 1—Spectrophotometric absorption curves of the colored products of triiodothyronine and thyroxine. Concentration: 4.5×10^{-5} M. Triiodothyronine: —; thyroxine: - - -

given by three noniodinated amino acids, dihydroxyphenylalanine, tryptophan, and tyrosine. Tryptophan gives a measurable color only in a much greater quantity (1 mg.) than would be present in samples used in their method, and tyrosine gives only about $1/80$ the color intensity of diiodotyrosine. They also reported that monoiodotyrosine does not give a color with nitrous acid and ammonia. Contrary to their findings we found that: (a) dihydroxyphenylalanine gives an amber color; (b) tryptophan gives no observable color even in greater quantity (ten times optimal concentration); (c) tyrosine gives no observable color; and (d) monoiodotyrosine gives an orange color. Further investigation of the color obtained with dihydroxyphenylalanine showed that the same color is produced when the amino acid is dissolved in concentrated ammonium hydroxide alone. Therefore, the coloration of this substance is probably due to formation of a quinoid structure under conditions of alkaline oxidation and not to any reaction with nitrous acid. During the addition of the nitrous acid no color formation was observed, whereas a yellow color is always obtained with iodinated tyrosines and 3,5-diiodothyronines.

Besides using this method for the determination of thyroxine in tablets, Adamson, *et al.* (7), found that 3,5-diiodothyronine gives about 65% of the color intensity of thyroxine when subjected to the color reaction. On the other hand, we obtained no color with diiodothyronine. Since our method is a virtual duplication of theirs, this difference is not attributable to experimental conditions. It may involve compound purity, and in this connection, it is noteworthy that 3,5-diiodothyronine and tyrosine are similar in that no iodine is present on the hydroxylated benzene ring; therefore, no reaction with nitrous acid would be expected.

Plati and Wenner (9) used this color test to determine the completeness of extraction of 3,5,3'-triiodothyronine from a crude triiodothyronine containing 10–15% thyroxine as an impurity in a continuous countercurrent solvent extraction column. They reported a red color as a positive test for 3,5,3'-triiodothyronine. This is in disagreement with our results shown in Table I. We obtained an orange color corresponding to the orange color obtained with monoiodotyrosine, a molecule of related structure. It appears that the orange color is associated with an *ortho* iodo-phenolic hydroxyl relationship on an aromatic amino acid. It is predicted that 3'-iodothyronine, 3,3'-diiodothyronine, or any 3'-iodinated thyronine will give the same color.

REFERENCES

- (1) Kendall, E. C., and Osterberg, A. E., *J. Biol. Chem.* 40, 265 (1919).
- (2) Ingvaldsen, T., and Cameron, A. T., *Trans. Roy. Soc. Can.*, 17, 20, 297 (1926).
- (3) Morton, M. E., and Chaikoff, I. L., *J. Biol. Chem.* 147, 1 (1943).
- (4) Roche, J., and Michel, R., *Compt. rend.*, 221, 521 (1945).
- (5) Roche, J., and Michel, R., *Ann. pharm. franc.*, 4, 1 (1946).
- (6) Roche, J., and Michel, R., *Biochim. et Biophys. Acta*, 1, 335 (1947).
- (7) Adamson, D. C. M., Domleo, A. P., Jefferies, J. P., and Shaw, W. H. C., *J. Pharm. and Pharmacol.*, 4, 760 (1952).
- (8) Gross, J., and Pitt-Rivers, R., *Biochem. J.*, 53, 645 (1953).
- (9) Plati, J. T., and Wenner, W., *U. S. Pat.* 2,784,222.

Properties and Applications of Powdered Polysaccharide Acids IV.*

Comparison of Carboxymethylcellulose (HMC) and Cornstarch as Tablet Disintegrants

By LLOYD KENNON† and JOSEPH V. SWINTOSKY†

Sulfathiazole tablets were prepared through a compressional force range of 0.25 to 2 tons per tablet. Tablet hardness and disintegration time were compared for carboxymethylcellulose and cornstarch disintegrants through a concentration range of 1 to 30%. The results indicate that hardness and disintegration times were similar for both disintegrants, except at the 30% starch concentration where tablet hardness was very low. Generally, hardness increased rapidly with increase in compressional force and was not depressed appreciably by increased disintegrant concentrations. Disintegration times decreased rapidly with increased disintegrant concentrations. The principal advantages of HMC in this formulation are that it can be granulated wet with the other tablet components without losing its disintegrant properties upon drying, also, wet granulations employing it dry faster than those without it, and the resultant tablets are somewhat harder than the corresponding tablets containing starch as the disintegrant.

IN A PREVIOUS PUBLICATION the preparation and properties of carboxymethylcellulose (HMC) obtained by ion exchange of sodium carboxymethylcellulose followed by spray drying were described (1). Possible areas of pharmaceutical usefulness of this product have been noted (2-4). The present study was undertaken to compare the disintegrant properties of this material to cornstarch in sulfathiazole tablets. An effort was made to determine the interrelationships of such variables as compressional force, hardness, disintegrant concentration, and disintegration time.

The literature describes a comparison of potato starch and alginic acid as disintegrating agents (5). Also a number of publications have defined the problems, described the equipment, and suggested methods for studying the static and dynamic factors which affect tablet formulation and manufacture (3, 5-27).

EXPERIMENTAL

Source of HMC and Starch.—The method of preparation of the HMC by ion exchange was modified from the previous procedure (1). Eight 4,000 cc Erlenmeyer flasks, each containing 1 Kg of acid charged sulfonic acid cation exchange resin, Amberlite IR 120 (Rohm & Haas, Philadelphia, Pa.) were employed. Three liters of 2% CMC, Premium Low Viscosity Type 70 (Hercules Powder Co., Wilmington, Delaware), in aqueous solution was added to flask 1, shaken periodically for fifteen to twenty minutes, then decanted into flask 2 after the resin settled. The process was repeated serially

until transfer through the eight flasks was complete. About 10 liters of solution were made before it was necessary to wash and recharge the resin. A white powder was obtained by spray drying the HMC solution as previously described (1).

A food grade Argo cornstarch was used in this study.

Preparation of the Granulations.—The granulation for the starch containing formulations was made by adding 30 Gm of 10% starch paste to each 97 Gm of sulfathiazole, U S P XIII powder (Mallinckrodt). The mixed mass was then pushed through a U S No 10 mesh stainless steel wire sieve, and the resulting granules were dried at 45° for four hours. The dried granules were then pushed through a No 20 mesh sieve and collected on a No 60 mesh sieve. The granules used to make tablets were those which passed through the former, but not the latter. These granules, containing 97% sulfathiazole, constituted the master granulation. Portions of it were used to make seven other granulations simply by mixing with proper amounts of dry starch powder to get granules for compression containing 1%, 2%, 4%, 8%, 15%, 30%, and 60% of dry starch.

The material for compression containing HMC was made by adding the HMC to the sulfathiazole powder, mixing, adding starch paste with some additional water, granulating, and drying. Granule size was standardized with No 20 and No 60 mesh sieves also. The preparation of the six HMC-sulfathiazole granulations is summarized in Table I.

The granulations containing 15% and 30% HMC become slightly tan during the drying process. This appeared to have been caused either by an unnecessarily protracted drying time or by a slightly excessive drying temperature. These granulations dried very rapidly.

Compressing the Tablets.—Seven sulfathiazole granulations employing dry starch powder as disintegrant and six employing HMC as the disintegrant were used for compression into tablets. Portions of 0.330 Gm each were weighed and com-

* Received August 30, 1957 from the laboratories of the University of Wisconsin School of Pharmacy, Madison.

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TABLE I.—COMPOSITION OF HCMC-SULFATHIAZOLE GRANULATIONS

Granulation No. →	1	2	3	4	5	6
HCMC, per cent	1	2	4	8	15	30
Sulfathiazole, Gm.	96	95.1	93.1	89.2	82.4	67.9
10% Starch paste, Gm.	30	29	29	28	26	21
HCMC, Gm.	1	2	4	8	15	30
Additional water, cc.	14	14	23	27	36	60
Drying time, hr. 45°	2	2	2	2	2	1.5

pressed into tablets using a special $\frac{3}{8}$ -inch punch and die set and a mechanical lever machine of a type previously described (6). The seven compressional forces used were 500, 1,000, 1,500, 2,000, 2,500, 3,000, and 4,000 pounds per tablet. The 4,000-lb. level corresponds to a pressure of 18 tons per sq. in. Good tablets are often prepared industrially at pressures of approximately 10 tons per square inch (28). Hence this report deals with a range that is significant in actual tablet manufacturing. Each of the 13 granulations was compressed into tablets at the seven force levels mentioned. Twenty tablets from each of the starch-sulfathiazole granulations and 12 tablets from each of the HCMC-sulfathiazole granulations were punched. Half of each separate group of tablets was used for the disintegration time determinations and half for the hardness tests.

The granulation containing 60% dry starch was not amenable to compression into tablets even under a compressional force of 10,000 lb.

Hardness Test.—Tablet hardness was determined with the Strong-Cobb tablet hardness tester (7, 29).

Disintegration Time Determination.—The method used for determining the disintegration times of the tablets was essentially that of the U. S. P. XIV (30) with the following modifications. The basket rack contained four compartments instead of the six recommended in the U. S. P. Water was used as the disintegration fluid, and its temperature was maintained at 37°. The submerged basket rack was raised and lowered through approximately a 5-cm. range 31 times a minute. A tablet was considered disintegrated when all of its fragments passed through the No. 8 mesh screen floor of its compartment in the basket rack.

RESULTS AND DISCUSSION

As shown in Fig. 1, scale readings of the Strong-Cobb hardness tester are linear with respect to pressure as determined with a compression tank and gauge. The results of the disintegration time and hardness tests are shown graphically in Figs. 2-5. Median values were used in this study. They were used because the number of tablets in each group tested was relatively small so that the occasional widely variant value encountered would affect the mean to an extent not commensurate with its own significance.

The probable errors figured for all the data taken on the basis of mean values were between 2% and 15%. Since median (not mean) values were used for the graphs, the errors should be less than the calculated ones because the occasional widely divergent results are eliminated.

Observation of Figs. 2 and 3 indicates increased disintegration time as the compressional force used

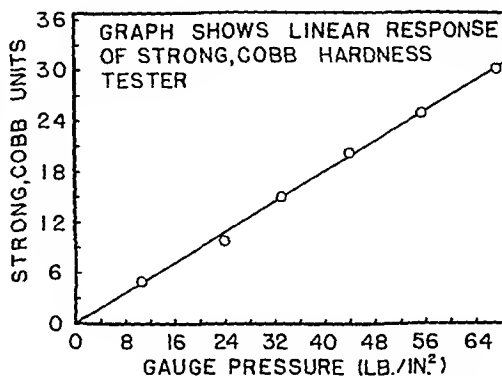


Fig. 1.—Plot shows the result of calibrating the Strong-Cobb hardness tester with a tank of compressed air.

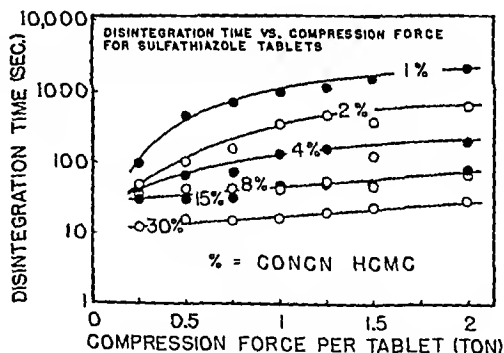


Fig. 2.—Graph shows the effect of compressional force on the disintegration time of sulfathiazole tablets containing various percentages of HCMC as disintegrant.

to make the tablets is increased. It is seen that the time of disintegration decreases greatly as the percentage of the disintegrant is increased. As the compressional forces are increased there is little difference between the disintegration times of the tablets containing 15% and 30% starch or HCMC.

In Figs. 4 and 5 it is observed that hardness generally increases quite rapidly with increase in compressional force. These figures are shown in the form of swath-graphs because the hardness values covered by the swath are primarily a function of the compressional force and appear to be much less dependent on disintegrant concentration. Hence it is not helpful to trace the individual lines which

cross and intermingle frequently. In general, however, tablet hardness appeared to decrease with disintegrant concentration, at least at the higher levels. This is most evident at the 30% starch concentration where tablets were relatively soft at

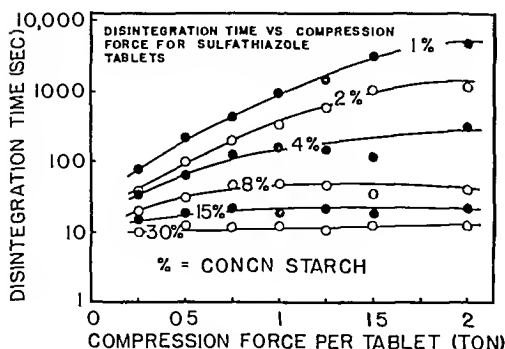


Fig 3—Plot illustrates the effect of compressional force on the disintegration time of sulfathiazole tablets containing various percentages of starch as disintegrant

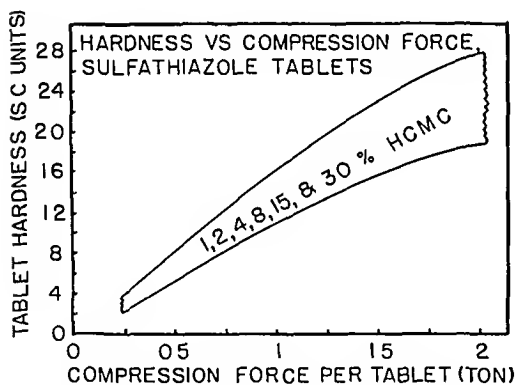


Fig 4—Plot indicates the effect of compressional force on the hardness of sulfathiazole tablets containing various amounts of HPMC

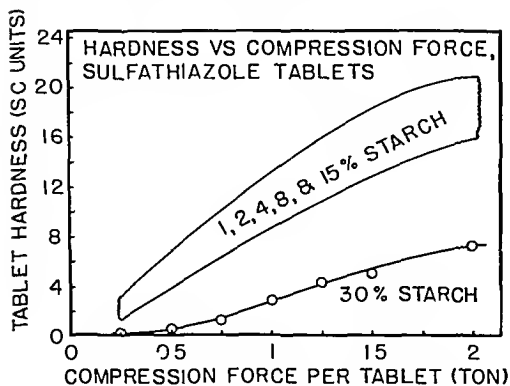


Fig 5—Graph demonstrates the effect of compressional force on the hardness of sulfathiazole tablets containing various amounts of starch

all compressional forces because of the poor compressibility of this component

The principal advantages of HPMC in this sulfathiazole formulation are: (a) It can be granulated wet with the other tablet ingredients without losing its disintegrant properties upon drying, hence, an extra weighing and mixing step in the tablet manufacturing procedure is eliminated, (b) wet granulations employing HPMC dry faster than the same granulations without it; (c) since HPMC is granulated along with the other ingredients, the tablets containing HPMC as the disintegrant are somewhat harder than the corresponding tablets containing starch, because no extra "fines" are added to the former. Starch, however, has advantages when the choice of a disintegrant is considered solely on the basis of cost and availability.

A comparison of all the data indicates that there is no great difference between HPMC and starch as regards hardness and disintegration times of sulfathiazole tablets where they are used as disintegrants. These and previously published results (2, 3, 5) are in concordance with the view that numerous polysaccharide acids and synthetic cation exchangers may be expected to possess useful tablet disintegrant properties by virtue of their swelling in water. This study is suggestive of a procedure that may be used for evaluating such or other new materials for utility as tablet disintegrants as they become available.

REFERENCES

- (1) Swintosky, J. V., and Kennon, L., *THIS JOURNAL*, 42, 505 (1953)
- (2) Swintosky, J. V., Kennon, L., and Tingstad, J., *ibid*, 44, 109 (1955)
- (3) Swintosky, J. V., Kennon, L., and Tingstad, J., *ibid*, 44, 112 (1955)
- (4) Swintosky, J. V., and Kaufman, A. M., *ibid*, 44, 540 (1955)
- (5) Berry, H., and Ridout, C. W., *J. Pharm. and Pharmacol.*, 2, 619 (1950)
- (6) Higuchi, T., Arnold, R. D., Tucker, S. J., and Busse, L. W., *THIS JOURNAL*, 41, 93 (1952)
- (7) Higuchi, T., Rao, A. N., Busse, L. W., and Swintosky, J. V., *ibid*, 42, 104 (1953)
- (8) Higuchi, T., Nelson, E., and Busse, L. W., *ibid*, 43, 344 (1954)
- (9) Nelson, E., Naqvi, S. M., Busse, L. W., and Higuchi, T., *ibid*, 43, 596 (1954)
- (10) Higuchi, T., Lowe, L. N., and Busse, L. W., *ibid*, 43, 685 (1954)
- (11) Elowe, L. N., Higuchi, T., and Busse, L. W., *ibid*, 43, 718 (1954)
- (12) Nelson, E., Busse, L. W., and Higuchi, T., *ibid*, 44, 223 (1955)
- (13) Nelson, E., *ibid*, 44, 494 (1955)
- (14) Strickland, W. A., Jr., Nelson, E., Busse, L. W., and Higuchi, T., *ibid*, 45, 51 (1956)
- (15) Nelson, E., *ibid*, 44, 435 (1955)
- (16) Stephenson, D., and Smith, D. S., *J. Pharm. and Pharmacol.*, 3, 547 (1951)
- (17) Howard, W. R., *ibid*, 3, 777 (1951)
- (18) Burlinson, H., and Pickering, C., *ibid*, 2, 630 (1950)
- (19) Berry, H., *Quart. J. Pharm. and Pharmacol.*, 12, 501 (1939)
- (20) Prance, H. P., Stephenson, D., and Taylor, A., *ibid*, 19, 286 (1946)
- (21) Berry, H., and Temple, E. M., *ibid*, 11, 364 (1938)
- (22) Berry, H., and Smith, A. N., *ibid*, 17, 248 (1944)
- (23) Smith, A. N., *Pharm. J.*, 163, 191, 477 (1949)
- (24) Smith, A. N., *ibid*, 164, 73, 132 (1950)
- (25) Smith, D. S., and Stephenson, D., *ibid*, 164, 139 (1950)
- (26) Gross, H. M., and Becker, C. H., *THIS JOURNAL*, 41, 157 (1952)
- (27) Ewe, G. E., *J. Am. Pharm. Assoc.*, 23, 1205 (1934)
- (28) Silver, J. A., and Clarkson, R., "Manufacture of Compressed Tablets," Stokes Machine Co., Philadelphia, 1944, p. 43
- (29) Hosler, W. H., *Bull. Natl. Formulary Comm.*, 18, 1 (1950)
- (30) "The Pharmacopoeia of the United States of America," 14th rev., Mack Publishing Co., Easton, Pa., 1950, p. 700

Synthesis and Antifungal Studies on New Organic Sulfur Compounds*

By AMRUTLAL M. SHAH and JAMES W. JONES†

Six derivatives of *o*-phenylenediamine-bis-dithiocarbamic acid and six derivatives of 4-chloro-*o*-phenylenediamine-bis-dithiocarbamic acid were prepared. Twelve new compounds, undecylenic acid and ethanol were screened for antimycotic activity against *M. Gypseum*, *M. Canis*, *T. Rubrum*, and *T. Mentagrophyte* by agar plate, paper-disk technique. All the compounds were more effective than undecylenic acid against all of the organisms with exception of the sodium salts of *o*-phenylenediamine-bis-dithiocarbamic acid which was more effective only against *M. Canis* and *T. Rubrum*.

VITAL STATISTICS reveals that fungal diseases of men are growing health problems (1). The search for antifungal agents has been in progress for several decades. Sulfur has long played an important role in the combat between humans and lower form of life as pests, fungi, and bacteria. Horsfall (2) reviewed the antimycotic therapy and presented a classification of antifungal agents. More recently organic sulfur compounds have been considered to be valuable as antifungal agents. Extensive researches have been carried out by plant pathologists for utilizing these organic sulfur compounds, dithiocarbamic acid and its derivatives, as agricultural fungicides (3,4). The above compounds showed remarkable antimicrobial activity for the treatment of fungus diseases of plants, however, very little attention has been given to their use in human chemotherapy.

Several esters of ethylene-bis-dithiocarbamic acid have been prepared in this laboratory and tested against human pathogenic fungi. Some of the compounds were found to be as active as undecylenic acid. The pungent odor and chemically unstable nature of these compounds is a hindrance to their pharmaceutical application (5).

In this investigation, an attempt has been made to prepare several esters of *o*-phenylenediamine-bis-dithiocarbamic acid and 4-chloro-*o*-phenylenediamine-bis-dithiocarbamic acid.

EXPERIMENTAL

Synthesis

A.—Disodium *o*-phenylenediamine-bis-dithiocarbamate, B.—Disodium 4-chloro-*o*-phenylenediamine-bis-dithiocarbamate.—One-eighth mole (13.5 Gm.) of *o*-phenylenediamine or 17.64 Gm. of 4-

chloro-*o*-phenylenediamine was dissolved, with the aid of heat, in 50 ml. of water in a three-necked, 500 ml. flask, fitted with a reflux, mercury-seal condenser and a dropping funnel. The solution was cooled to 30° and a solution of one-fourth mole (11 Gm.) of sodium hydroxide in 50-ml. of water was added in one lot. This mixture was stirred for fifteen minutes. One-fourth mole (22 Gm.) of carbon disulfide was then added dropwise, with stirring, over a period of thirty to forty minutes. The mixture was stirred at 30–40° for three and one-half hours. The solution was then evaporated to a small volume and an excess of acetone was added. This mixture was placed in a refrigerator until crystallization was complete. The crystals were purified by several recrystallizations from aqueous solution by the addition of acetone.

Esters of *o*-phenylenediamine-bis-dithiocarbamic acid and 4-chloro-*o*-phenylenediamine-bis-dithiocarbamic acid.—In preparing the esters, the sodium salts of the acids were prepared as described above except that the phenylenediamines were dissolved in alcohol instead of water. After the initial mixture had refluxed for three and one-half hours, one-fourth mole of alkyl halide was added over a period of fifteen minutes. The mixture was then refluxed, with stirring, for six hours, during which time a dark, oily or resinous mass separated. The oil or mass was collected by decanting the mother liquor and extracting the residue with ether. After evaporating off the ether, the residues were dried in a vacuum oven at 50° for twenty-four hours.

The yields and N analyses are shown in Table I. Mycological

The compounds synthesized were tested for antifungal activity by the procedure described by Klingman and Rosenweig (6) for the determination of fungicidal activity *in vitro*.

Table I lists the structural formulas and numbers of the compounds tested for antimycotic activity. The results of the mycological work are summarized in Tables II, III, IV, and V.

DISCUSSION

It was found that the sodium salts and the methyl and ethyl esters of the dithiocarbamic acids were unstable and decomposed at room temperature while the allyl, epoxypentyl, and benzyl esters were stable. According to Horsfall, *et al.* (7), decomposition may be due to formation of the corresponding isothiocyanates.

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Abstracted from a dissertation submitted to the Graduate College of the State University of Iowa by Amrutlal M. Shah in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE I—COMPOUNDS SYNTHESIZED AND/OR TESTED

	R	Yield, %	Calcd., %	Found, %
	$\begin{array}{c} \text{S} \quad \text{H} \quad \text{H} \quad \text{S} \\ \parallel \quad \diagdown \quad \diagup \quad \parallel \\ \text{R}-\text{S}-\text{C}-\text{N} \quad \text{---} \quad \text{N}-\text{C}-\text{S}-\text{R} \end{array}$			
1	Na—	40		
2	CH ₃ —	70	9.79	9.58
3	C ₂ H ₅ —	40	8.86	8.92
4	CH ₂ =CHCH ₂ —	50	8.21	8.15
5	CH ₂ =CH—CH ₂ — O	47	7.50	7.32
6	C ₆ H ₅ —CH ₂ —	37	6.34	6.41
	$\begin{array}{c} \text{S} \quad \text{H} \quad \text{H} \quad \text{S} \\ \parallel \quad \diagdown \quad \diagup \quad \parallel \\ \text{R}-\text{S}-\text{C}-\text{N} \quad \text{---} \quad \text{N}-\text{C}-\text{S}-\text{R} \end{array}$			
7	Na—	39		
8	CH ₃ —	69	8.66	8.50
9	C ₂ H ₅ —	76	7.98	7.89
10	CH ₂ =CH—CH ₂ —	55	7.46	7.31
11	CH ₂ =CH—CH ₂ — O	80	6.86	6.90
12	C ₆ H ₅ —CH ₂ —	80	5.89	5.71
13	Undecylenic Acid			
14	Ethanol			

The procedure used for testing antifungal activity does not give the inhibition value as an end point, which is the minimum concentration required to inhibit the growth of a certain species. The method gives a preliminary indication of the potential which a compound might possess in comparison to a standard antifungal agent which is used frequently, undecylenic acid.

The Tables II, III, IV, and V indicate that most of the compounds were active against one or more strains of fungi. One factor which influences the width of inhibition zone is the speed with which the organism grows. *M. Gypseum* and *T. Mentagrophyte* seem to grow considerably faster than *M. Canis* and *T. Rubrum*.

Compounds 1 and 7 are water-soluble sodium salts. Generally, they were less active than undecylenic acid against all 4 organisms. Compounds 2 and 8, the methyl esters, showed more activity than undecylenic acid against all 4 organisms. Kloppe and Van der Kerk (8) showed that optimum activity occurs with the lower alkyl esters, i. e., the methyl and ethyl esters of dithiocarbamic acids. The results in this work agree with that observation. Compounds 3 and 9, the ethyl esters, were considerably more active than undecylenic acid against all 4 organisms, less active than compounds 2 and 8 against *M. Gypseum* and *T. Mentagrophyte* and about equally as active against *M. Canis* and *T. Rubrum*.

TABLE II—ZONES OF INHIBITION IN MILLIMETERS AGAINST *M. GYPSEUM*

Compound No	1%	2%	3%	4%	5%
1	0	0	0	0.25	0.25
2	0.25	1.00	3.25	4.25	7.25
3	0	0.25	1.00	2.25	4.00
4	0	1.25	4.00	4.50	6.50
5	0	0	5.00	1.00	1.50
6	2.00	3.50	7.00	8.75	10.50
7	0	0	0	0	0
8	5.25	10.25	11.50	15.00	15.50
9	4.50	9.00	10.75	12.00	13.00
10	1.50	3.00	6.00	7.50	9.75
11	1.25	1.75	2.75	3.25	5.25
12	5.75	9.00	9.75	10.50	12.00
13	0	0	0.25	0.50	1.50
14	0	0	0	0	0

TABLE III—ZONES OF INHIBITION IN MILLIMETERS AGAINST *T. RUBRUM*

Compound No	1%	2%	3%	4%	5%
1	9.75	13.00	15.75	19.00	20.50
2	11.75	12.75	18.25	19.50	21.00
3	11.50	14.50	18.25	21.25	21.50
4	13.00	17.50	18.00	18.75	20.25
5	4.50	11.50	13.25	16.75	18.75
6	13.25	14.00	15.50	16.25	18.50
7	3.50	5.00	6.00	7.00	8.75
8	13.25	15.75	16.00	17.00	18.25
9	10.00	11.50	12.00	13.25	17.75
10	11.50	12.25	12.50	14.00	18.50
11	10.25	12.25	13.25	15.00	15.50
12	14.00	15.00	18.25	20.75	20.75
13	6.25	8.00	8.50	9.25	9.75
14	0	0	0	0	0

TABLE IV—ZONES OF INHIBITION IN MILLIMETERS AGAINST *M. CANIS*

Compound No	1%	2%	3%	4%	5%
1	9.50	11.00	13.00	14.25	17.50
2	10.00	13.00	15.50	16.00	21.00
3	9.75	11.75	15.25	17.00	18.00
4	8.00	11.50	12.50	13.00	14.25
5	3.00	4.25	6.25	8.25	9.50
6	9.50	10.75	11.00	13.50	14.50
7	0	2.00	3.00	3.25	4.00
8	7.50	12.75	13.50	16.75	18.25
9	8.75	13.50	15.25	17.00	18.00
10	8.00	9.50	13.50	15.00	15.25
11	9.00	9.50	10.00	10.50	11.50
12	12.25	13.25	14.25	16.00	17.75
13	2.25	3.25	5.50	7.25	7.50
14	0	0	0	0	0

TABLE V—ZONES OF INHIBITION IN MILLIMETERS AGAINST *T. MENTAGROPHYTE*

Compound No	1%	3%	3%	1%	5%
1	0	0	0	0	2.00
2	5.75	7.25	9.50	9.75	11.00
3	2.00	3.00	5.50	6.00	6.50
4	1.00	2.00	2.50	5.25	6.50
5	2.00	2.00	2.50	4.00	4.50
6	2.75	6.00	7.75	8.00	9.25
7	0	0	0	2.00	2.25
8	4.75	6.00	7.75	9.25	11.25
9	2.75	4.50	6.00	8.00	8.50
10	4.00	5.75	7.25	9.00	10.00
11	2.00	2.50	3.00	4.25	5.00
12	6.75	7.25	8.25	9.25	10.75
13	2.00	2.75	3.00	3.50	4.00
14	0	0	0	0	0

The decrease in activity might be due to the ester chain being increased with a consequent decrease in the extent of ionization. Compounds 4 and 10, the allyl esters, were considerably more active than undecylenic acid against all 4 organisms, but showed an activity which, in general, was less than that of the methyl esters. Again, a decrease in antifungal activity by lengthening the carbon chain in the ester part of molecule was shown. However, the allyl esters showed more activity than the epoxypropyl esters against all 4 organisms. According to Friedman (9), this greater activity may be due to the $-\text{CH}=\text{CH}-$ group. Compounds 6 and 12, the benzyl esters, were more inhibitory against all 4 species than the allyl or epoxypropyl esters but less inhibitory than the methyl esters with some exceptions. The greater activity over the allyl or epoxypropyl ester may be due to the fact that these compounds have a smaller number of aliphatic carbon atoms in the ester chain of molecule. The lesser activity of the benzyl esters than that shown by the methyl esters may be due to the higher molecular weight of the ester group, which may decrease the tendency of the compounds to ionize causing them to be less effective.

Compound 14, ethanol, showed no inhibition against any of the fungi used. It was used as a control since it was used to dissolve the esters.

In the past, many efforts have been made to change the molecular structure of this type of compounds with regard to the thiocarbamate portion and to compare their activities. The results of this investigation suggest that dithiocarbamic acid derivatives prepared from *o*-phenylenediamine and 4-chloro-*o*-phenylenediamine possess a higher antifungal activity than derivatives prepared from ethylenediamine.

REFERENCES

- (1) Reiss, F., "Annals of the New York Academy of Sciences, Medical Mycology," Vol 50, Art 10, 1950, p 1211
- (2) Horsfall, J G., "The Chromica Botanica Company," Waltham, Mass, 1956, p 141
- (3) Tisdale, W H., and Flemmer, A L., *Ind Eng Chem* 34, 50(1942)
- (4) U S pat 1,648,259
- (5) Collins, A F., Ph D Dissertation, State University of Iowa, 1956
- (6) Klingman, A M., and Rosenweig, W, *J Invest Dermatol*, 10, 59(1948)
- (7) Horsfall, J G., *Phytopathology*, 37, 2(1947)
- (8) Klopping, H L., and Van der Kerk, G J M., *Rec Trav Chim*, 70, 924(1951)
- (9) Friedman, H L., Natl Research Council (US) publication 206, Washington, D C, 1950

The Pharmacology of 4-[2-(5-Phenyloxazolyl)]-1-Methylpyridinium Chloride and *p*-Toluenesulfonate*

By THOMAS J. HALEY, ANNA M. FLESHER, and N. KOMESU

It has been shown that 4-[2-(5-phenyloxazolyl)]-1-methylpyridinium chloride or *p*-toluenesulfonate produced a dose dependent hypotension which could not be modified by atropine and pyrilamine maleate. There was no significant effect on respiration until exitus. Electrocardiographic changes indicate that these compounds are toxic to the heart. There was no evidence that the pyridinium compounds had any cholinergic, adrenergic or ganglionic blocking properties. Although the pyridinium compounds produced hypothermia, their potency was of a low order, while their toxicity was of a high order, thus preventing use in therapeutics. The pyridinium compounds do not induce or prevent chromoachrydemia in rats, but their vasodilator effect does prevent epinephrine death in mice.

RECENTLY, Lushbaugh, *et al* (1), reported that the oxazole quaternary salts produced poikilothermia in mice. Haley, *et al* (2), observed that these compounds also produced hypotension, but had no effect on autonomic ganglia. Ott, *et al* (3), synthesized the chloride and *p*-toluenesulfonate salts of a new compound, 4-

[2-(5-phenyloxazolyl)]-1-methylpyridine. The quaternary nitrogen is in the pyridyl rather than the oxazolyl ring (Fig. 1). We have investigated these compounds to determine what differences

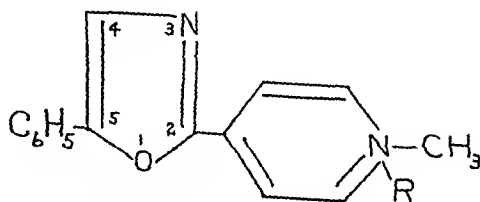


Fig 1.—Substituted quaternary pyridinium compounds: R = Cl or $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3$

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in pharmacological activity have been produced by such structural changes.

EXPERIMENTAL

Nineteen cats of both sexes, weighing 2.2 to 3.85 Kg., were anesthetized with 35 mg./Kg. of sodium pentobarbital intraperitoneally. Blood pressure was recorded from the carotid artery with a mercury manometer. Respiration was recorded by the method of Haley (4). *In situ* intestinal contractions were recorded with a balloon-tambour system. The ECG was obtained with Lead II of a Sanborn Viso-Cardiette. Ganglionic effects were studied by recording contractions of the nictitating membrane following stimulation of the preganglionic cervical sympathetic fibers with 8V/10 sec. from a Grass Model S-4 Stimulator. Similar stimulation of the vagus nerve was used to study anticholinergic activity. Five spinal cats were prepared in the usual manner. Doses of the drugs used were: pyridinium compounds, 0.5–15 mg./Kg., epinephrine, 3 mcg./Kg., acetylcholine, 5 mcg./Kg.; histamine, 0.1 mcg./Kg., atropine, 2 mg./Kg.; and pyrilamine maleate, 1 mg./Kg. The blocking drugs were given one-half hour prior to the pyridinium compounds and tests were made after each dose to establish the maintenance of the blockade throughout the experiment. Effects on body temperature were studied in CF-1 mice using electrical thermometers according to the method of Clark and Trolander (5). Cholinergic stimulant effects were studied using the chromoachrydymia response of the rat (5, 7). Effects on epinephrine mortality in mice were studied by the method of Loew and Micetich (8). Ocular effects were studied after topical application to the eyes of New Zealand rabbits.

RESULTS

Cardiovascular and Respiratory Effects.—Both pyridinium compounds produced a hypotension which depended upon the initial blood pressure and the dose administered, see Table I. A response plateau usually developed at the 10 mg./Kg. dose level. With most animals, there was an abrupt hypotensive response followed by a progressive continuous decrease in mean arterial pressure.

The duration of effect varied with each dose and usually was of the order of fifteen to twenty minutes at the 5 mg./Kg. dose. The pyridinium compounds appeared to have some cumulative action, possible on receptors in the peripheral vascular system, because there was a tendency to bleed from the incisions associated with the insertion of the various recording devices. This effect became pronounced after the 5 mg./Kg. dose at which time the animals had received a total dose of 8.5 mg./Kg. Based on an equivalent hypotension, acetylcholine is 1,000 times and histamine 5,000 times more active than the pyridinium compounds. Neither atropine nor pyrilamine maleate was able to block the pyridinium compound induced hypotension, although the former blocked the acetylcholine response and the latter blocked the histamine response. However, they did reduce the progressive decrease in blood pressure which followed the initial abrupt fall. This stabilization of the blood pressure resulted in greater responses at the higher doses. In the spinal cat, both pyridinium compounds produced their usual hypotensive response.

Neither pyridinium compound had any great effect on respiration except to produce a slight transient increase in depth coincident with the hypotension. Cheyne-Stokes respiration occurred sporadically in several animals. Death was due to respiratory failure coupled with cardiovascular collapse.

Effects on the Electrocardiogram.—Both pyridinium compounds had a progressive negative chronotropic effect on the cat heart *in situ*, finally reducing the heart rate 40 to 60 beats/minute. This effect was not modified by atropine and pyrilamine maleate. There was a transient increase in the height of the T-wave with each dose of pyridinium compounds, indicative of cardiac anoxia. Of a more serious nature was the observation of auricular fibrillation, sinus arrhythmia, trigeminy, ventricular tachycardia, left bundle branch block and left ventricle strain. These electrocardiographic changes were observed in the majority of animals and were only partially blocked by atropine and pyrilamine maleate. At exitus all cats showed auriculoventricular dissociation, but this occurred only after respiratory paralysis.

Effects on the Autonomic Nervous System.—Neither compound had any effect on the response

TABLE I.—HYPOTENSIVE EFFECTS OF QUATERNARY PYRIDINIUM COMPOUNDS

Compound	Dose, mg./Kg.	Hypotension in Millimeters of Mercury—	
		No. Blocking Drug, Mean \pm S. E.	Atropine and Pyrilamine Maleate, Mean \pm S. E.
4-2-(5-phenyloxazolyl)-1-methylpyridinium chloride	0.5	47 \pm 1.2	26 \pm 3.8
	1.0	51 \pm 3.1	43 \pm 2.4
	2.0	74 \pm 4.1	71 \pm 4.1
	5.0	82 \pm 3.6	103 \pm 7.2
	10.0	71 \pm 6.4	100 \pm 6.5
	15.0		63 \pm 9.0
4-2-(5-phenyloxazolyl)-1-methylpyridinium- p-toluenesulfonate	0.5	41 \pm 4.04	27 \pm 1.0
	1.0	42 \pm 4.09	35 \pm 2.7
	2.0	38 \pm 2.95	44 \pm 6.0
	5.0	77 \pm 10.28	88 \pm 5.5
	10.0	81.5 \pm 11.3	88 \pm 7.5
	15.5	76 \pm 13.9	91 \pm 9.3

of the nictitating membrane to preganglionic stimulation; also they did not block the response produced by intravenous epinephrine or acetylcholine. The pyridinium compounds did not cause contraction of the nictitating membrane at any of the doses used. No adrenergic blocking or atropine-like effects of the pyridinium compounds were observed when epinephrine or acetylcholine was administered intravenously. Furthermore, the effects of vagal stimulation on the heart, blood pressure, and intestinal motility were not inhibited by the pyridinium compounds.

Effects on Intestinal Motility in Situ.—Both compounds increased the tonus, amplitude, and rate of contraction of the cat intestine at all doses used and the maximum effect was obtained at 5 mg./Kg. Such effects were greater than those produced by acetylcholine and had a longer duration. The intestinal relaxation produced by epinephrine was not affected by the pyridinium compounds.

Effects on Body Temperature.—Administration of 100 mg./Kg. of the methochloride derivative reduced mouse body temperature from the control value of 30° to 25.5° within three hours, but four of five mice died. With the methyl-*p*-toluenesulfonate derivative, a dose of 200 mg./Kg. killed six of six mice over a period of five hours, thus preventing any evaluation of hypothermic effect. A dose of 150 mg./Kg. reduced body temperature from the control 30° to 26.2° six hours after injection, at

ium compounds were greatly depressed, quiescent, and cold to the touch. The compounds also appear to have a low renal threshold because they rapidly appeared in the urine giving it their characteristic fluorescent yellow color. It was quite evident that the methochloride derivative was more toxic than the methyl-*p*-toluenesulfonate derivative and that both compounds become more toxic after the epinephrine injection, see Table II.

A profuse chromoachryodinia was produced in rats by 10 mg./Kg. of acetyl- β -methylcholine but neither pyridinium compound was capable of either initiating or blocking this effect at doses of 100, 250, or 500 mg./Kg. Furthermore, the two higher doses were lethal within five to ten minutes and the smaller dose shortly after the test dose of acetyl- β -methylcholine.

DISCUSSION

The substitution of the pyridyl group for the 1-naphthyl, 4-methylphenyl or 4-methoxyphenyl group in position 2 of the oxazole ring with the quaternary nitrogen in the pyridyl group, rather than in the oxazolyl group, increases the overall toxicity and decreases the hypothermic activity of the compound. This also introduces a series of detrimental myocardial effects which would prevent any application of the pyridinium compounds in human therapeutics. In all other aspects, both series of compounds appear to produce almost equivalent pharmacological effects. In so far as their hypotensive actions are con-

TABLE II.—EPINEPHRINE TOXICITY ANTAGONISM BY PYRIDINIUM COMPOUNDS

Compound	I. P. Dose, mg./Kg.	Total Mortality by Hours			
		1	5	7	24
Saline 0.9%	1 ml.	7/10	8/10	8/10	9/10
4-2-(5-phenyloxazolyl)-1-methylpyridinium chloride	75	0/10 ^a	0/10	1/10	1/10
		0/10	2/10	5/10	10/10
	100	1/10 ^a	4/10	4/10	5/10
		0/10	6/10	10/10	10/10
4-2-(5-phenyloxazolyl)-1-methylpyridinium- <i>p</i> -toluenesulfonate	75	0/10 ^a	0/10	0/10	0/10
		0/10	0/10	2/10	8/10
	100	0/10 ^a	0/10	0/10	1/10
		0/10	1/10	5/10	10/10

^a Control no epinephrine, all other mice received 14 mg./Kg. of epinephrine hydrochloride intravenously thirty minutes after the pyridinium compound.

which time three of six animals died. The 100 mg./Kg. dose was not lethal but reduced body temperature only 0.5°.

Miscellaneous Effects.—After topical application of 1% solutions of the pyridinium compounds to the eyes of rabbits, there was no evidence of irritation, local anesthesia or changes in pupillary size.

Prior intraperitoneal administration of the pyridinium compounds prevented death from intravenous epinephrine under the conditions specified by Loew and Micetich (8), see Table II. This protective action was probably related to the vasodilation produced by the pyridinium compounds because these compounds are devoid of adrenergic blocking activity. In this regard the results were similar to those observed with acetyl- β -methylcholine (8). All of the mice receiving the pyridin-

ium compounds were greatly depressed, quiescent, and cold to the touch. The compounds also appear to have a low renal threshold because they rapidly appeared in the urine giving it their characteristic fluorescent yellow color. It was quite evident that the methochloride derivative was more toxic than the methyl-*p*-toluenesulfonate derivative and that both compounds become more toxic after the epinephrine injection, see Table II.

REFERENCES

- (1) Hayes, F. N., Langham, W. H., *J. Pharmacol. Exptl. Therap.*, 116,
- (2) Haley, I. J., McCormick, W. G., and Flesher, A. M., *Arch. intern. Pharmacodynam.*, 109, 78(1957).
- (3) Ott, D. G., Hayes, F. N., and Kerr, V. N., *J. Am. Chem. Soc.*, 78, 1941(1956).
- (4) Haley, T. J., *THIS JOURNAL*, 37, 73(1948).
- (5) Clark, L. C., Jr., and Trolander, H., *J. Am. Med. Assoc.*, 155, 251(1954).
- (6) Winbur, and Hambourger, *W. B., J. Phar*, 1940.
- (7) Burger, *J. Phar*, 4, 185(1919).
- (8) Loew, E. R., and Micetich, A., *J. Pharmacol. Exptl. Therap.*, 93, 434(1918).

Chemical Composition of Gum Turpentine of Pines XXIX.*

A Report on *Pinus ponderosa* from Five Localities: Central Idaho, Central Montana, Southeastern Wyoming, Northwestern Nebraska, and Central Eastern Colorado

By N. T. MIROV and P. M. ILOFF, Jr.

Five samples of *Pinus ponderosa* turpentine were analyzed. The sample from southwestern Idaho was composed of: *l*- α -pinene, 6 per cent; *l*- β -pinene, 30 per cent; β -myrcene, 3 per cent; *d*- Δ^3 -carene, 40 per cent; *l*,*dl*-limonene, 10 per cent; terpinolene, 2 per cent; and cadinenene, 5 per cent. The central Montana sample contained: *l*- α -pinene, 3 per cent; *l*- β -pinene, 25 per cent; β -myrcene, 5 per cent; *d*- Δ^3 -carene, 47 per cent; *l*,*dl*-limonene, 6 per cent; terpinolene, 4 per cent; longifolene, 2 per cent; and cadinenene, 5 per cent. The sample from southeastern Wyoming was composed of *l*- α -pinene, 2 per cent; *l*- β -pinene, 31 per cent; β -myrcene, 4 per cent; *d*- Δ^3 -carene, 40 per cent; *l*,*dl*-limonene, 4 per cent; terpinolene, 1 per cent; longifolene, 10 per cent; and cadinenene, 5 per cent. Southwestern Nebraska turpentine contained: *l*,*dl*- α -pinene, 2 per cent; *l*- β -pinene, 32 per cent; β -myrcene, 3 per cent; *d*- Δ^3 -carene, 38 per cent; *l*,*dl*-limonene, 7 per cent; longifolene, 12 per cent; and cadinenene, 4 per cent. The sample from southeastern Colorado was composed of: *dl*- α -pinene, 5 per cent; *l*- β -pinene, 17 per cent; β -myrcene, 10 per cent; *d*- Δ^3 -carene, 40 per cent; *l*,*dl*-limonene, 15 per cent; and longifolene, 8 per cent.

THE CHEMICAL COMPOSITION of turpentine of *Pinus ponderosa* from several localities has been reported in previous articles of the present series. These localities were: Sierra Nevada of California, coastal mountains of California, northern Idaho, Utah, Arizona, Black Hills of South Dakota, and Rocky Mountains of central Colorado (2-5). They represent only part of the tree's natural range. *P. ponderosa* occurs from the Pacific Coast to Nebraska and from the southern part of western Canada to the Mexican border. Therefore it appeared desirable to analyze turpentines of this pine from some additional, "strategically" situated places.

This paper reports the composition of turpentine obtained from five other localities; southwestern Idaho, central Montana, southeastern Wyoming, northwestern Nebraska, and southeastern Colorado.

EXPERIMENTAL

Turpentine was obtained by heating, under reduced pressure, the oleoresin samples from these five localities. At the end of each operation, when all the turpentine was recovered, the temperature of the pot residue (rosin) was increased to 200° and the pressure was reduced to 0.1 mm.

Pinus ponderosa from Southwestern Idaho.—A

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sample of oleoresin was collected near Idaho City, Idaho, at an elevation of 4,070 feet.

The turpentine amounted to 26.5% of the weight of oleoresin and had the following physical characteristics: d_4^{20} , 0.8563; n_D^{20} , 1.4758; $[\alpha]_D^{20}$ — 7.2. A 404-Gm. batch of turpentine was distilled at a pressure of 11 mm. in a Todd column, 0.25 mm. inside diameter, packed with $\frac{3}{32}$ -inch glass helices, and equipped with a Todd automatic reflux head. A reflux ratio of 10:1 was maintained.

We had learned previously that the bulk of *P. ponderosa* turpentine from any locality consists predominantly of Δ^3 -carene and varying amounts of α - and β -pinenes. Accordingly, the percentage of α -pinene, β -pinene and Δ^3 -carene were determined by physical properties alone, using Sutherland's method (7).

We estimated that the turpentine sample of *Pinus ponderosa* from southwestern Idaho contained 6% of *l*- α -pinene (fractions 1 to 5), 30% of *l*- β -pinene (fractions 6 to 10), and 40% *d*- Δ^3 -carene (fractions 11 to 14).

A slight change in physical properties of Fraction 12 indicated the possible presence of a substance other than Δ^3 -carene. This fraction was analyzed for β -myrcene using the method of Berry (1). Ten grams of the oil and 0.8 Gm. of maleic anhydride were heated for thirty minutes at 100°. The solution was cooled, 20 ml. of hexane was added, and the resulting mixture was kept at —5° for two days. The resulting crystals were recrystallized from hexane twice, and 0.44 Gm. of crystals were received. The crystals possessed a melting point of 33.5 to 34.5°. The melting point was not depressed by admixture of maleic anhydride adduct of known β -myrcene.

The physical properties of fraction 19 indicated a probable presence of *l*,*dl*-limonene. A 2-ml. sample of the fraction was treated with bromine according

to the method of Berry (1). After one month of refrigeration, the reaction mixture was filtered and 50 mg. of crude tetrabromide was received. The tetrabromide was recrystallized twice from ethyl acetate, after which it melted at 124–125°. The melting point was not depressed when an authentic *d,l*-limonene tetrabromide was admixed.

Fraction 24 had physical properties which indicated the presence of terpinolene. Two grams of the fraction were treated with bromine using the procedure of Berry (1); 2.60 Gm. of crude tetrabromide were obtained. After six recrystallizations from ethyl acetate a tetrabromide, m. p. 116–117°, was received. The melting point was not depressed by admixture with authentic terpinolene tetrabromide.

Fraction 28 consisted mainly of sesquiterpenes and had the following properties: d_4^{25} , 0.9159; n_D^{25} , 1.5031; $[\alpha]_D^{25}$ – 33.4. This fraction was redistilled over sodium at 14 mm. pressure; all fractions had negative rotations. A heart cut had the following properties: b_{14} 130–135°, d_4^{26} , 0.9104; n_D^{25} , 1.5036; $[\alpha]_D^{25}$ – 30.1.

A 3-ml. portion of the heart cut was used to prepare 200 mg. of a hydrochloride which after three recrystallizations from glacial acetic acid had a melting point of 120–123.5°. Admixture of *l*-cadinene hydrochloride did not depress the melting point.

To sum up, *P. ponderosa* turpentine from southwestern Idaho was composed of *l*- α -pinene, 6%; *l*- β -pinene, 30%; *d*- Δ^3 -carene, 40%; β -myrcene, 3%; *l,d*-limonene, 10%; terpinolene, 2%; and cadinene, 5%.

***Pinus ponderosa* from Central Montana.**—The Montana sample of turpentine was distilled from oleoresin collected near the town of White Sulphur Springs, along the course of Whitetail Deer Creek, at an elevation of 5,000 feet.

The yield of turpentine was 17.7%; its physical characteristics were these: d_4^{25} , 0.8614; n_D^{25} , 1.4760; $[\alpha]_D^{25}$ – 0.9. A 330-Gm. batch was distilled through the Todd column, described above, at 10 mm. pressure. A reflux ratio of 10:1 was maintained.

Sutherland's (7) density/index of refraction diagram indicated that fractions 1 and 2 consisted mainly of *l*- α -pinene (about 3% of the total). Fractions 3 to 10 were those of *l*- β -pinene (25%). Fractions 11 to 14 contained *d*- Δ^3 -carene (47%). Fraction 11 contained about 5% of β -myrcene. This open-chain terpene was identified by preparing a maleic anhydride adduct, according to Berry (1). Admixture of known maleic anhydride adduct of myrcene did not depress the melting point of the substance.

A rapid increase of *levo*-rotation of the following fractions, peaking in fraction 19, caused us to suppose that fractions 15 to 21 contained *l,d*-limonene.

Two milliliters of fraction 19 were treated with bromine by the method of Berry (1); 100 mg. of crude tetrabromide were received. After three recrystallizations from ethyl acetate the tetrabromide melted at 124.5° and was not depressed in melting point by admixture of authentic *d,l*-limonene tetrabromide.

Fraction 22 had properties indicating the presence of terpinolene. Two grams of the fraction were treated with bromine using the procedure of Berry (1); 2.75 Gm. of crude tetrabromide were obtained.

After four recrystallizations from ethyl acetate, 0.31 Gm. of tetrabromide, m. p. 116–117°, was received. The melting point was not depressed by admixture of authentic terpinolene tetrabromide. About 8% of the charge boiled above 105° at 10 mm.; it consisted mainly of sesquiterpenes. The properties of the sesquiterpene fractions indicated that there were apparently two sesquiterpenes, which occurred in the highest purity in fraction 28 and fraction 29.

Fraction 28 had the following physical properties: d_4^{25} , 0.9321; n_D^{25} , 1.5120; $[\alpha]_D^{25}$ +36.3.

A 3-ml. portion of fraction 28 was used to prepare a hydrochloride. After two recrystallizations from glacial acetic acid, 0.79 Gm. of crystals were received; the crystals melted at 58.5–59°. Admixture of known hydrochloride of *d*-longifolene showed no depression.

Fraction 29 had the following physical properties: d_4^{25} , 0.9159; n_D^{25} , 1.5052; $[\alpha]_D^{25}$ –28.2. A 3-ml. portion of fraction 29 was used to prepare 0.33 Gm. of hydrochloride. After two recrystallizations from glacial acetic acid, 72 mg. of hydrochloride (m. p. 120–121°; n_D^{25} –41, c = 1.32 in chloroform) were received. Admixture of known *levo*-rotatory cadinene hydrochloride did not depress the melting point.

To sum up, turpentine obtained from *Pinus ponderosa* growing in central Montana contained: *l,d*- α -pinene, 3%; *l*- β -pinene, 25%; β -myrcene, 5%; *d*- Δ^3 -carene, 47%; *l,d*-limonene, 6%; terpinolene, 4%; longifolene, 2%; cadinene, 5%.

***Pinus ponderosa* from Southeastern Wyoming.**—The material for our work was obtained in the Laramie Mountains near a place called Esterbrook, at an elevation of 6,500 feet. The yield of turpentine was 22.0% of the weight of the oleoresin.

The turpentine had the following characteristics: d_4^{25} , 0.8673; n_D^{25} , 1.4768; $[\alpha]_D^{25}$ +1.5.

A 416-Gm. batch was distilled through the previously mentioned Todd column at 10 mm. pressure. A reflux ratio of 10:1 was maintained.

Composition of fractions 1 to 14 was determined by physical characteristics, according to the method proposed by Sutherland (7), except for the identification of β -myrcene.

Fraction 1 consisted chiefly of *l*- α -pinene (2%); fractions 2 to 8 contained *l*- β -pinene (31 per cent). Fractions 9 to 14 were *d*- Δ^3 -carene fractions (40% of the total oil). Fraction 10 was analyzed for β -myrcene.

Using Berry's procedure (1), 0.40 Gm. of maleic anhydride adduct, m. p. 33.5 to 34.5°, was produced. The melting point of the adduct was not depressed by admixture of the known maleic anhydride adduct of myrcene. Myrcene amounted to about 4% of the total oil.

The physical properties of fraction 17 indicated the probable presence of *l,d*-limonene. A 1.5-ml. sample of the oil was dissolved in 1:1 ethanol-ethyl ether and treated at 0° with bromine according to the method of Wallach (8). The solvent was evaporated, and the oil was mixed with methanol. After several days of standing at –5°, the crystals were filtered out and recrystallized three times from ethyl acetate. Sixty milligrams of tetrabromide, m. p. 124–125°, were received. A mixed melting with authentic *d,l*-limonene tetrabromide showed no depression. Limonene amounted to 4% of the total oil.

TABLE I —FRACTIONAL DISTILLATION OF PINUS PONDEROSA TURPENTINE FROM FIVE DIFFERENT LOCALITIES

Fractions	Pressure, mm	Boiling Range, °C	Distillate, %	Density	Index of Refraction	Specific Rotation
<i>Pinus ponderosa</i> from Idaho City, Idaho (404 Gm used)						
				d_4^{25}	n_D^{25}	$[\alpha]_D^{25}$
1	11	40-42	1 9	0 8540	1 4647	-21 0
2	11	42-44	1 0	0 8547	1 4661	-21 1
3	11	44-45	1 2	0 8573	1 4680	-22 2
4	11	45-46	1 0	0 8573	1 4701	-22 2
5	11	46-47	0 8	0 8588	1 4710	-23 0
6	11	47-49	3 9	0 8573	1 4727	-18 5
7	11	49-51	4 0	0 8521	1 4717	- 8 2
8	11	51	5 1	0 8525	1 4712	- 0 9
9	11	51-52	6 9	0 8536	1 4714	- 2 3
10	11	52-53	9 8	0 8510	1 4718	- 0 4
11	11	53	12 2	0 8499	1 4711	+ 5 9
12	11	53-54	8 4	0 8485	1 4706	+11 3
13	11	54	10 5	0 8503	1 4707	+13 4
14	11	54-55	10 0	0 8536	1 4708	+11 4
15	11	55-56	4 2	0 8544	1 4712	- 4 4
16	11	56-57	1 1	0 8507	1 4720	-34 8
17	11	57-58	1 1	0 8444	0 4726	-55 0
18	11	58-59	0 7	0 8433	1 4729	-71 4
19	11	59-60	1 8	0 8426	1 4732	-84 7
20	11	60-63	0 9	0 8426	1 4749	-78 7
21	11	63-66	0 7	0 8470	1 4791	-44 4
22	11	66-67	0 5	0 8536	1 4823	-20 1
23	11	67-68	1 1	0 8562	1 4847	- 6 8
24	11	68-69	1 2	0 8559	1 4847	- 1 2
25	11	69-82	0 7	0 8596	1 4807	- 4 0
26	11	82-92	0 8	0 9208	1 4959	- 7 2
27	11	92-103	1 1	0 9513	1 5100	0 0
28	11	Above 103	5 2	0 9159	1 5031	-33 4
Residue and losses			3 2			
<i>Pinus ponderosa</i> from White Sulfur Springs, Montana (330 Gm used)						
				d_4^{25}	n_D^{25}	$[\alpha]_D^{25}$
1	10	39-41	1 6	0 8577	1 4669	-16 9
2	10	41-42	1 6	0 8595	1 4690	-18 9
3	10	42-43	2 3	0 8610	1 4720	-21 2
4	10	43-44	4 0	0 8625	1 4719	-21 8
5	10	44-45	1 2	0 8632	1 4746	-23 8
6	10	45-46	2 5	0 8632	1 4760	-23 3
7	10	46-47	3 0	0 8625	1 4762	-21 3
8	10	47-48	3 8	0 8606	1 4756	-15 3
9	10	48-49	2 7	0 8566	1 4744	- 8 2
10	10	49-50	4 6	0 8544	1 4738	- 2 1
11	10	50-51	12 0	0 8518	1 4728	+ 5 2
12	10	51-51 5	13 0	0 8521	1 4720	+10 2
13	10	51 5-52	13 0	0 8540	1 4718	+14 1
14	10	52-53	11 5	0 8569	1 4720	+11 6
15	10	53-54	1 0	0 8540	1 4730	- 7 2
16	10	54-55	0 7	0 8507	1 4738	-32 9
17	10	55-56	0 8	0 8485	1 4741	-36 9
18	10	56-58	0 7	0 8448	1 4751	-50 8
19	10	58-60	0 5	0 8448	1 4756	-56 1
20	10	60-64	0 7	0 8477	1 4798	-40 7
21	10	64-66	0 8	0 8532	1 4841	-11 3
22	10	66-67	2 1	0 8547	1 4850	-16 4
23	10	67-74	4 2	0 8628	1 4770	- 8 1
24	10	74-90	1 5	0 9185	1 4878	- 8 4
25	10	90-105	0 3	0 9381	1 4898	- 3 4
26	10	105-110	0 6	0 9392	1 4840	+ 4 0
27	10	110-115	1 8	0 9358	1 4940	+25 4
28	10	115-116	1 3	0 9321	1 5120	+36 3
29	10	Above 116	3 7	0 9159	1 5052	-28 2
Residue and loss			2 5			
<i>Pinus ponderosa</i> from Esterbrook, Wyo (416 Gm used)						
				d_4^{25}	n_D^{25}	$[\alpha]_D^{25}$
1	10	42-44	2 0	0 8580	1 4670	-12 1
2	10	44-46	1 3	0 8654	1 4700	-16 2
3	10	46-47	2 1	0 8591	1 4722	-18 6
4	10	47-48	6 2	0 8606	1 4737	-18 8
5	10	48-49	4 5	0 8588	1 4738	-15 8

TABLE I.—(Continued)

Fractions	Pressure, mm.	Boiling Range, ° C.	Distillate, %	Density	Index of Refraction	Specific Rotation
6	10	49-50	4.2	0.8580	1.4735	-11.4
7	10	50-51	6.0	0.8555	1.4730	- 6.4
8	10	51-52	6.5	0.8544	1.4723	- 0.9
9	10	52-53	9.7	0.8521	1.4718	+ 4.7
10	10	53	8.0	0.8525	1.4711	+10.2
11	10	53	10.2	0.8533	1.4709	+13.3
12	10	53-54	1.4	0.8577	1.4708	+15.3
13	10	54	9.7	0.8555	1.4709	+15.2
14	10	54-55	3.0	0.8558	1.4716	+10.1
15	10	55-56	1.0	0.8566	1.4728	- 1.0
16	10	56-57	0.6	0.8525	1.4740	-14.1
17	10	57-59	0.4	0.8492	1.4751	-24.5
18	10	59-62	0.4	0.8470	1.4769	-25.4
19	10	62-65	0.5	0.8485	1.4797	-22.1
20	10	65-67	0.5	0.8529	1.4821	-13.6
21	10	67-68	1.2	0.8548	1.4845	- 3.2
22	10	68-70	1.2	0.8548	1.4831	- 0.3
23	10	70-80	0.3	0.8622	1.4784	- 3.4
24	10	80-92	0.6	0.9086	1.4861	- 7.8
25	10	92-105	1.0	0.9433	1.4918	- 3.6
26	10	105-112	1.0	0.9418	1.4858	+ 4.2
27	10	112-115	3.6	0.9381	1.4901	+20.5
28	10	115-117	1.6	0.9344	1.4978	+35.8
29	10	117-118	3.5	0.9326	1.5019	+40.7
30	10	118-119	1.2	0.9311	1.5027	+35.4
31			4.1	0.9134	1.5052	-40.5
Pot residue and loss			2.5			
<i>Pinus ponderosa</i> from Chadron, Nebraska (365 Gm. used)						
			d_{25}^{25}	$n_D^{25.5}$	$[\alpha]_D^{25}$	
1	11	42-44	1.8	0.8551	1.4660	- 2.4
2	11	44-46	1.7	0.8548	1.4681	- 7.2
3	11	46-47	1.9	0.8557	1.4693	- 9.3
4	11	47-48	3.2	0.8563	1.4709	-11.7
5	11	48-49	5.0	0.8557	1.4720	-12.2
6	11	49-50	6.0	0.8560	1.4728	-11.8
7	11	50-51	4.2	0.8541	1.4726	- 8.8
8	11	51-52	6.5	0.8519	1.4725	- 6.0
9	11	52-53	5.2	0.8510	1.4720	- 2.8
10	11	53-54	9.9	0.8494	1.4712	+ 4.2
11	11	54-55	12.2	0.8488	1.4702	+10.5
12	11	55	12.0	0.8517	1.4701	+12.9
13	11	55-56	3.6	0.8532	1.4708	+ 4.6
14	11	56-57	1.4	0.8507	1.4725	-10.3
15	11	57-58	1.0	0.8488	1.4724	-27.7
16	11	58-59	0.5	0.8454	1.4731	-40.4
17	11	59-61	0.5	0.8435	1.4740	-48.7
18	11	61-63	0.6	0.8425	1.4753	-51.4
19	11	63-66	0.4	0.8457	1.4781	-40.7
20	11	66-68	0.8	0.8497	1.4817	-16.7
21	11	68-69	1.3	0.8530	1.4832	- 3.8
22	11	69-75	0.8	0.8508	1.4802	- 1.2
23	11	75-90	0.5	0.9176	1.4828	- 3.7
24	11	90-93	0.4	0.9292	1.4921	- 4.8
25	11	93-106	1.0	0.9389	1.4930	+ 0.2
26	11	106-112	0.6	0.9393	1.4981	+13.4
27	11	112-116	1.3	0.9358	1.4902	+24.3
28	11	116-118	2.3	0.9302	1.4973	+39.2
29	11	118-119	4.8	0.9273	1.5011	+40.0
30	11	119	2.0	0.9258	1.5020	+28.8
31	11	Above 119	3.9	0.9135	1.5042	-24.0
Pot residue and losses			2.7			
<i>Pinus ponderosa</i> from Beulah, Colorado (420 Gm. used)						
			d_{25}^{25}	n_D^{25}	$[\alpha]_D^{25}$	
1	10	39-40	0.6	0.8550	1.4642	+ 1.1
2	10	40-41	3.3	0.8561	1.4657	- 2.3
3	10	41-43	2.9	0.8575	1.4683	- 6.5
4	10	43-45	1.4	0.8598	1.4703	-11.6
5	10	45-46	3.5	0.8617	1.4723	-13.9
6	10	46-47	1.4	0.8620	1.4732	-14.4
7	10	47-48	4.6	0.8639	1.4738	-13.0

TABLE I.—(Continued)

Fractions	Pressure, mm.	Boiling Range, ° C.	Distillate, %	Density	Index of Refraction	Specific Rotation
8	10	48-49	3.2	0.8595	1.4734	- 8.2
9	10	49-50	2.3	0.8580	1.4734	- 4.9
10	10	50-51	2.9	0.8587	1.4730	- 0.5
11	10	51-52	8.9	0.8569	1.4726	+ 4.1
12	10	52	10.0	0.8573	1.4721	+ 8.3
13	10	52-53	9.2	0.8573	1.4718	+11.2
14	10	53	10.0	0.8584	1.4715	+14.3
15	10	53	10.0	0.8588	1.4713	+ 9.2
16	10	53-54	2.1	0.8595	1.4721	-15.1
17	10	54-56	1.5	0.8573	1.4730	-42.7
18	10	56-57	0.8	0.8451	1.4747	-63.7
19	10	57-59	1.0	0.8440	1.47742	-75.9
20	10	59-63	0.8	0.8492	1.4770	-63.7
21	10	63-65	0.5	0.8496	1.4808	-35.1
22	10	65-66	0.4	0.8647	1.4837	-17.7
23	10	66-67	1.3	0.8695	1.4855	- 4.6
24	10	67-68	0.7	0.8566	1.4833	- 1.4
25	10	68-80	1.0	0.8617	1.4881	- 6.1
26	10	80-93	0.9	0.9015	1.4801	- 8.6
27	10	93-106	0.8	0.9347	1.4863	- 0.6
28	10	106-112	0.8	0.9381	1.4875	+12.7
29	10	112-114	0.9	0.9347	1.4941	+32.0
30	10	114-115	0.6	0.9318	1.4981	+39.8
31	10	115-116	2.7	0.9322	1.5018	+46.4
32	10	116	2.3	0.9325	1.5028	+46.1
33	10	Above 116	3.1	0.9218	1.5045	+ 6.3
Pot residue and loss			2.8			

Fraction 21 had physical properties which indicated the presence of terpinolene. Two grams of the fraction were treated with bromine using the procedure of Berry (1). 2.53 Gm. of crude tetrabromide were obtained. After four recrystallizations from ethyl acetate, 0.45 Gm. of crystals, m. p. 116-117°, were received. The melting point was not depressed by admixture of known terpinolene tetrabromide. Terpinolene amounted only to 1% of the total oil. About 15% of the original charge boiled above 105° at 10 mm. and consisted mainly of sesquiterpenes. Distillation data indicated the presence of two sesquiterpenes, which occurred in highest concentration in fraction 29 and fraction 31.

Fraction 29 had the following physical properties: d_{20}^{25} , 0.9326; n_D^{25} , 1.5019; $[\alpha]_D^{25}$ +40.7. A 3-ml. portion of fraction 29 was used to prepare a hydrochloride. After two recrystallizations from glacial acetic acid, 1.2 Gm. of crystals which melted at 59 to 59.5° were received. An admixture of authentic hydrochloride of *d*-longifolene did not depress the melting point of the hydrochloride.

Fraction 31 had the following physical properties: d_{20}^{25} , 0.9134; n_D^{25} , 1.5052; $[\alpha]_D^{25}$ -40.5. A 3-ml. portion of fraction 31 was used to prepare 0.41 Gm. of hydrochloride. After two recrystallizations from glacial acetic acid, 53 mg. of hydrochloride (m. p. 119-119.5°; α_D^{25} -39, c = 1.05 in chloroform) were received. An admixture of authentic cadinene hydrochloride did not depress the melting point.

To sum up, turpentine from the Laramie Mountains, southeastern Wyoming, contained: *l*- α -pinene, 2%; *l*- β -pinene, 31%; β -myrcene, 4%; *d*- Δ^3 -carene, 40%; *l*,*dl*-limonene, 4%; terpinolene, 1%; longifolene, 10%; cadinene, 5%.

Pinus ponderosa from Northwestern Nebraska. Oleoresin was obtained from trees growing at the easternmost locality of distribution of *P. ponderosa*, near the town of Chadron, Nebraska, at an elevation of 3,500 feet. The oleoresin yielded 18.9% of turpentine, which had the following physical characteristics: d_{20}^{25} , 0.8695; n_D^{25} , 1.4781; $[\alpha]_D^{25}$ -0.9°. A 3.65-Gm. batch of turpentine was distilled through the Todd column at 11 mm. pressure. A reflux ratio of 10:1 was maintained.

Fractions 1 to 13, as in the previous samples, were studied by means of the Sutherland *d/n* diagram (7).

Fractions 1 and 2 consisted of *l*- α -pinene (2% of the total oil). Fractions 3 to 9 contained mainly *l*- β -pinene (32%). Fractions 11 to 13 were those of α - Δ^3 -carene (38%). β -Myrcene (3%) was identified in fraction 11 by preparing a maleic anhydride adduct, according to the method outlined by Berry (1).

The physical properties of fraction 18 indicated the probable presence of *l*,*dl*-limonene. Two grams of the oil were treated with bromine, as outlined previously (1). The twice recrystallized tetrabromide had a melting point of 124.5°, which was not depressed by admixture of authentic *dl*-limonene tetrabromide.

The properties of fraction 21 indicated the presence of terpinolene. The fraction was redistilled over sodium at atmospheric pressure. Two milliliters of a heart cut were treated with bromine using the procedure of Berry (1); 4.39 Gm. of crude tetrabromide were received. After four recrystallizations from ethyl acetate, 0.36 Gm. of a tetrabromide, m. p. 117.5 to 118.5°, was received. The

melting point was depressed by admixture with known terpinolene tetrabromide but the melting point was not depressed on admixture of the tetrabromide (m p 117.5 to 118.5°) obtained from fraction 20 of the turpentine from Beulah, Colorado (see next sample)

It should be noted that both fraction 23 of *P. ponderosa* from Beulah, Colorado, and fraction 21 of *P. ponderosa* from Chadron, Nebraska, were redistilled at atmospheric pressure over sodium. This is probably the reason for the discrepancy in results.

About 15% of the original charge boiled above 106° at 11 mm and consisted mainly of sesquiterpenes. The distillation data indicated the presence of two sesquiterpenes, which occurred in highest concentration in fraction 29 and fraction 31.

Fraction 29 (17.6 Gm) had the following properties: d_4^{20} , 0.9273; n_D^{25} , 1.5011; $[\alpha]_D^{25}$, +40.0. A 3 ml portion of fraction 29 was used to prepare a hydrochloride. After two recrystallizations from glacial acetic acid, 1.3 Gm of crystals melting at 58 to 59° were received. An admixture of authentic *d* longifolene hydrochloride showed no melting point depression.

Fraction 31 (14.4 Gm) had the following properties: d_4^{20} , 0.9135; n_D^{25} , 1.5042; $[\alpha]_D^{25}$, -24.0. A 3 ml portion of fraction 31 was used to prepare 0.51 Gm of crude hydrochloride. After two recrystallizations from glacial acetic acid, 70 mg of hydrochloride (m p 119 to 120°, α^{25} , -40, c = 1.36 in chloroform) were received. When known cadinene hydrochloride of negative rotation was added, the melting point was not depressed.

To sum up, turpentine obtained from northwestern Nebraska, contained 1, *dl* α pinene, 2%, 1 β pinene, 32%, β myrcene, 3%, *d* Δ^3 carene, 38%, 1, *dl* limonene, 7%, longifolene, 12%, cadinene, 4%.

Pinus ponderosa from Southeastern Colorado.—The oleoresin was collected near the settlement of Beulah, some 35 miles southwest of Pueblo, Colorado. Elevation of the place was 7,500 feet. Upon distillation, the oleoresin yielded 20.0% of turpentine. The turpentine possessed the following physical properties: d_4^{22} , 0.8661; n_D^{22} , 1.4760; $[\alpha]_D^{22}$, +1.8°. A 420-Gm batch of turpentine was distilled in the above described Todd column at 10 mm pressure. A reflux ratio of 10:1 was maintained.

Physical properties of fractions 1 to 15 were studied following Sutherland's method (7). Fractions 1 to 3 consisted of slightly dextrorotatory α pinene, (about 5%), apparently with an admixture of 1 α pinene. Fractions 4 to 9 were 1- β pinene fractions (about 17%). Fractions 12 to 15 were composed of *d*- Δ^3 carene (about 40%). Fractions 10 and 11 apparently contained β myrcene.

Berry's procedure (1) was carried out with 10 Gm of maleic anhydride and 10 Gm of fraction 11, 150 mg of adduct, m p 33.5 to 34.5°, were obtained. The melting point was not depressed by admixture with the maleic anhydride adduct of known β myrcene.

A sudden change in rotation in fraction 16 and a rapid increase in a levorotatory compound, with a peak of -64.0 in fraction 19, indicated the presence of 1, *dl* limonene in fractions 16 to 22. Two milliliters of the fraction were treated by the method of Berry (1). 1.14 Gm of crude tetrabromide were

received. After several recrystallizations from ethyl acetate, alternated with chloroform and addition of cold methanol, a few mg of crystals melting at 103 to 104° were received. The melting point was not depressed by admixture of authentic *dl*-limonene tetrabromide. Limonene amounted to 15% of the total oil.

Fraction 23 had properties which indicated the presence of terpinolene. The fraction was redistilled at atmospheric pressure over sodium. Two milliliters of a heart cut were treated with bromine using the procedure of Berry (1). 3.04 Gm of crude tetrabromide were received. After four recrystallizations from ethyl acetate, 0.47 Gm tetrabromide, m p 117.5 to 118.5°, was received. The melting point was depressed by admixture with either terpinolene tetrabromide or the tetrabromide of 1, β phellandrene. Apparently, terpinolene was absent in this sample. About 11% of the original charge boiled above 106° at 10 mm pressure and consisted mainly of sesquiterpenes. The distillation data indicated that the main sesquiterpene constituent was most pure in fraction 31.

Fraction 31 had the following physical properties: d_4^{24} , 0.9322; n_D^{23} , 1.5018; $[\alpha]_D^{24}$, +46.4. A 3 ml portion of fraction 31 was used to prepare a hydrochloride. After two recrystallizations from glacial acetic acid, 1.6 Gm of crystals which melted at 58.5 to 59° were received. When authentic longifolene was added, the melting point was not depressed.

Fraction 33 had the following physical characteristics: d_4^{24} , 0.9218; n_D^{23} , 1.5045; $[\alpha]_D^{24}$, +6.3. The fraction was redistilled over sodium, producing a 7.0 Gm heart cut with the properties: d_4^{23} , 0.923; n_D^{23} , 1.5039; $[\alpha]_D^{23}$, +13.7. A 3 ml portion of the heart cut was used to prepare a hydrochloride. After two recrystallizations from glacial acetic acid, 0.21 Gm of hydrochloride, m p 59 to 59.5°, was received. The melting point was not depressed by admixture of authentic hydrochloride of *d* longifolene.

To sum up, the turpentine sample from southeastern Colorado was composed of the following ingredients: *dl* α pinene, 5%, 1 β pinene, 17%, β myrcene, 10%, *d* Δ^3 carene, 40%, 1, *dl* limonene, 15%, longifolene, 8%.

The results of the analyses of the five samples of turpentines, together with seven samples previously reported (2-5) show that *Pinus ponderosa* turpentine, throughout the extensive range of the distribution of this species, always contained large amounts of Δ^3 carene, within the species there are several chemical varieties or forms. Penfold (6) calls these physiological varieties. In some of these varieties, monoeleic terpenes were represented by limonene, in others by limonene and terpinolene.

In some varieties the sesquiterpenes were represented by longifolene and cadinene, in other varieties by cadinene alone or by longifolene alone.

REFERENCES

- (1) Berry, P. A. *Australian Chem. Inst. J. & Proceed.* 14, 37 (1947).
- (2) Haagen-Smith, A. J., Redemann, C. T., Wang, T. H., and Murov, N. T. *This Journal*, 39, 260 (1950).
- (3) Hoff, P. M., Jr., and Murov, N. T. *ibid.* 43, 373 (1954).
- (4) Murov, N. T. *ibid.* 40, 410 (1951).
- (5) Murov, N. T., and Hoff, P. M., Jr. *ibid.* 45, 153 (1956).
- (6) Penfold, A. R. *Australasian J. Pharm.*, 16, 168 (1935).
- (7) Sutherland, M. D. *U. of Queensland Papers Dept. of Chem.* 1, No. 31 (1938).
- (8) Wallach, O. *Ann.* 225, 318 (1881).

Composition of Gum Turpentine of Pines XXX*

A Report on *Pinus serotina*, *Pinus tenuifolia*, and *Pinus yunnanensis*

By N. T. MIROV

Pinus serotina turpentine was found to consist of *l*- α -pinene, 5 per cent; *l*-limonene, 90 per cent; methyl chavicol, 1 per cent; and 2 or 3 per cent of unidentified sesquiterpenes. *P. tenuifolia* turpentine contained: *d*- α -pinene, 77 per cent; *l*- β -pinene, 6 to 7 per cent; Δ^3 -carene, 8 per cent; terpinolene, 1 to 2 per cent; methyl chavicol (possibly) less than 1 per cent; linalool, about 1 per cent; sesquiterpene fraction, 1 per cent. *P. yunnanensis* turpentine contained *l*- α -pinene, 87 per cent; possibly small amounts of *l*- β -pinene; oxygenated terpene compounds, 5 or 6 per cent. Apparently there were no sesquiterpenes.

Pinus Serotina Mich.—*Pinus serotina*, or pond pine, sometimes is described as a subspecies (1) or a variety of *Pinus rigida*. Shaw (7) and Little (5) consider this pine as an independent species.

Pinus serotina grows on the coastal plains from southern New Jersey to central and northwestern Florida and Alabama. Its turpentine was analyzed in 1908 by Herty and Dixon (3). The steam distilled turpentine had these physical characteristics: density, $d_{20}^{20} = 0.8478$; index of refraction, $n_D^{20} = 1.4734$; specific rotation, $[\alpha]_D^{20} = -105.36'$. The turpentine began to boil at 172° . In the fraction boiling at 175° to 176° , Herty and Dixon identified *l*-limonene by preparation of a tetrabromide. No other terpenes were identified, although a small amount of sesquiterpenes was suspected. Herty and Dixon reported that 90 per cent of the turpentine consisted of *l*-limonene.

In 1954 we received a sample of pond pine oleoresin from the Olustee Experimental Forest, northern Florida. The turpentine was obtained by heating the oleoresin under reduced pressure; at the end of the operation, the pot temperature reached 212° , and the pressure was reduced to 2 mm. The yield of turpentine was 19 per cent of the weight of the oleoresin. The physical characteristics of the turpentine were these: density, $d_{25}^{25} = 0.8437$; index of refraction, $n_D^{25} = 1.4716$; specific rotation, $[\alpha]_D^{25} = -83.7$.

A 300-Gm. batch of the turpentine was fractionated with a Vigreux-type column, 80 cm. long and 1.5 cm. inside diameter, equipped with a heated jacket. A reflux ratio 10 to 1 was maintained. The pressure was 663 mm. In fractions 1 and 2, α -pinene was identified. A copious precipitate of pinene nitrosochloride was obtained from both fractions when the oil was diluted with glacial acetic acid, chilled, mixed with amyl nitrite, and a 1:1 mixture of hydrochloric and acetic acids was added dropwise. After three recrystallizations from chloroform by addition of cold methanol, the nitrosochloride melted at 104 – 105° .

Attempts to identify β -pinene in fraction 6 were not successful. Physical characteristics of fractions

8 to 17 (Table I) clearly indicated the presence of large amounts of *l*-limonene. This terpene was identified in fraction 13 by preparation of limonene tetrabromide. After four recrystallizations from ethyl acetate, the tetrabromide possessed a melting point of 105 – 106° . Fraction 17 was extremely fragrant. It contained a great deal of limonene, to be sure, but also, apparently, an admixture of some oxygenated compounds. This fraction was redistilled to remove most of limonene. The higher boiling part of the fraction (1.3 Gm.) was oxidized to homoanistic acid. [See THIS JOURNAL 43, 741 (1954)]. Recrystallized from hot *n*-hexane, the homoanistic acid possessed a m. p. of 85 – 86° . The homoanistic acid was further oxidized, by the action of chronic anhydride, to anistic acid. Recrystallized from *n*-hexane, the acid had a melting point of 183.7 – 184.6° . There was no depression of melting point upon admixture of authentic anistic acid. Thus, presence of methyl chavicol in the turpentine of *P. serotina* was established.¹ Possibly the flask residue contained some sesquiterpenes, but these did not amount to more than 2 or 3% of the total oil.

To sum up, turpentine of *P. serotina* contained: *l*- α -pinene, 5%; *l*-limonene, 90%; methyl chavicol, 1%, and 2 or 3% of unidentified sesquiterpenes.

Pinus Tenuifolia Benth.—*Pinus tenuifolia* Benth. was discovered and named in 1839, but later it lost its specific rank and was described by Shaw (7) as a variety of *Pinus pseudostrobus*. Martinez (6) considers it an independent species.

Judging by the chemistry of its turpentine, which is much more complicated and quite different from that of *P. pseudostrobus*, we concur with Martinez, and consider *P. tenuifolia* a valid species.

An oleoresin sample of *P. tenuifolia* came to us through the courtesy of Mr. Louis Huguet from the vicinity of Uruapan, Michoacan, Mexico. The turpentine was expelled from the oleoresin under reduced pressure; at the end of the distillation, pot temperature was 180° , and pressure was 2 mm. The yield of turpentine was 27%. The physical characteristics of the turpentine were as follows: density, $d_{25}^{25} = 0.8600$; index of refraction, $n_D^{25} = 1.4672$; specific rotation, $[\alpha]_D = +25.6$.

A batch of 964 Gm. of the turpentine was fractionated in the Todd apparatus described in previous articles of this series; 72% of the turpentine was

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Included in this article is a brief summary of the entire project.

¹ Thanks are due to Dr. Gene Kritchevsky for identification of methyl chavicol.

distilled under atmospheric pressure, the rest under a pressure of 9 mm. Hg. The results of the fractional distillation are shown in Table I.

The lower-boiling fractions consisted of *d*- α -pinene; this terpene was identified in fraction 2 by the usual preparation of nitrosochloride, which after several precipitations from chloroform by cold

methanol, possessed a melting point of 105 to 106°. Fraction 12 was tested for β -pinene, by the usual preparation of nopinic acid, which, after two recrystallizations from benzene, had a melting point of 127°.

Fraction 16 yielded a nitrosate; after recrystallization from chloroform by addition of cold methanol,

TABLE I.—FRACTIONAL DISTILLATION OF TURPENTINE OF *PINUS SEROTINA*, *P. YUNNANENSIS*, AND *P. TENUIFOLIA*

Fractions	Pressure	Boiling Range, °C.	Distillate, %	Density	Index of Refraction	Specific Rotation
(1) <i>Pinus serotina</i> (308 Gm. used)						
1	663	150-161	1.7	0.8445	n_D^{25} 1.4617	$[\alpha]_D^{25}$ -42.0
2	663	161-162	4.0	0.8416	1.4681	-49.8
3	663	162-163	1.9			
4	663	163-164	9.5	0.8423	1.4689	-57.9
5	663	164-165	2.2			
6	663	165-166	14.6	0.8426	1.4693	-70.6
7	663	166-167	1.3			
8	663	167-168	2.2	0.8402	1.4702	-88.0
9	663	168-169	16.6	0.8397	1.4706	-100.0
10	663	169-170	4.7			
11	663	170-171	5.5	0.8396	1.4709	-108.4
12	663	171-172	7.8			
13	663	172-173	14.6	0.8396	1.4714	-117.3
14	663	173-174	2.3			
15	663	174-175	1.3	0.8427	1.4725	-111.7
16	663	175-178	1.7			
17	663	178-200	1.9	0.8713	1.4829	-85.0
Residue	4.9
Losses	1.3
(2) <i>Pinus tenuifolia</i> (964 Gm. used)						
1	760	107-155	0.4	d_4^{25} 0.8557	n_D^{25} 1.4641	$[\alpha]_D^{25}$ +40.4
2	760	155-156	62.0	0.8558	1.4651	+35.1
3	760	156-157	9.7	0.8569	1.4662	+29.9
4	760	38-39	2.6	0.8579	1.4673	+22.8
5	769	39-41	1.1	0.8588	1.4681	+18.9
6	769	41-43	0.4	0.8593	1.4697	+8.2
7	769	43-44	0.8	0.8595	1.4710	-0.2
8	769	44-45	0.5	0.8595	1.4721	-7.0
9	769	45-46	0.8	0.8602	1.4733	-13.7
10	769	46-47	0.9	0.8602	1.4740	-17.0
11	769	47-48	1.0	0.8602	1.4740	-17.0
12	769	48-49	1.2	0.8602	1.4731	-4.6
13	769	49-50	1.3	0.8584	1.4719	+3.5
14	769	50-52	1.4	0.8581	1.4712	+13.5
15	769	52-53	1.2	0.8581	1.4730	+14.5
16	769	53-54	3.5	0.8508	1.4750	+7.4
17	769	54-55	2.3	0.8548	1.4800	-0.9
18	769	55-56	0.5	0.8614	1.4843	-2.1
19	769	56-62	0.7	0.8626	1.4850	-0.9
20	769	62-66	0.6	0.8711	1.4782	-1.2
21	769	66-68	0.5	0.8711	1.4681	-2.5
22	769	68-70	0.3	0.8711	1.4685	-12.1
23	769	70-80	0.4	0.8964	1.4745	-12.0
24	769	80-84	0.6	0.9433	1.4841	-9.4
25	769	84-86	0.5	-8.9
26	769	86-90	0.3
27	769	90-95	0.8
Residue and losses	3.7
(3) <i>Pinus yunnanensis</i> (29 Gm. used)						
1	760	153-154	3.8	d_4^{25} 0.8535	n_D^{25} 1.4630	$[\alpha]_D^{25}$ -47.3
2	760	154-155	61.3	0.8510	1.4638	-50.5
3	760	155-157	13.1	0.8523	1.4648	-47.7
4	760	157-159	5.9	0.8556	1.4664	-42.1
5	760	159-161	2.4	0.8530	1.4683	-32.2
Residue	...	above 161	7.6	0.8743	1.4823	-6.9
Losses	5.9

the nitrosate melted at 146°. Thus the presence of *d*- Δ^3 -carene in turpentine of *P. tenuifolia* was established.

Terpinolene was detected in fraction 21. A tetrabromide prepared from the oil of this fraction was recrystallized from chloroform by addition of cold methanol. The melting point of the tetrabromide was 109 to 110°.

Fractions 23 and 24 possessed a fragrant odor, suggesting the presence of an oxygenated terpene compound. Fraction 24 was examined by Dr. Gene Kritchevsky and was found to contain linalool. Its *p*-nitrobenzoate possessed a melting point of 69.0 to 70°. Its phenylurethane melted at 63.6 to 64.2°. The spectral data of fraction 24 suggested the presence of small amounts of methyl chavicol.

Judging from the density of fraction 27, it obviously contained some sesquiterpenes. A hydrochloride was prepared using 3 Gm. of the oil of this fraction, dissolved in 24 cc. of dry ether, and treated with dry HCl gas for thirty minutes at 0°. The ether was then evaporated. Upon standing in a refrigerator for three weeks, hydrochloride crystals were formed, but these melted as soon as the evaporating dish was removed from the refrigerator.

Similar behavior of a hydrochloride melting at low temperature has been observed in *Pinus insularis* and *P. glabra*.

To sum up, turpentine of *P. tenuifolia* contained *d*- α -pinene, 77%; *l*- β -pinene, 6 to 7%; *d*- Δ^3 -carene, 8%; terpinolene, 1 to 2%; methyl chavicol (possibly), less than 1%; linalool, about 1%; sesquiterpene fraction, 1%.

Pinus yunnanensis Franchet.—*Pinus yunnanensis* was described by Franchet in 1899 as an independent species. Shaw (8) considers it as a variety and calls it *Pinus sinensis* var. *yunnanensis*. Later, however, the name *P. sinensis* has fallen into misuse and to a certain degree has been replaced by the name *tabulaeformis*. *Pinus yunnanensis* has again become in the eyes of some botanists (4) a valid species.

Wu (9), working with herbarium material, arrived at the conclusion that *Pinus yunnanensis* and *Pinus insularis* are the same species. It is seen then that opinions differ as to the botanical status of *P. yunnanensis*.

This pine grows in all of Yunnan province except the alpine zone and in the southwestern part of Kweichow province. It also occurs in upper Burma, northern Viet Nam, and in a narrow belt of northern India.

As it was difficult to obtain oleoresin of this pine from its native habitat, the writer tapped a few planted trees growing in the Eddy Arboretum, Institute of Forest Genetics, near Placerville, California. A little more than 30 Gm. of turpentine was obtained by heating the oleoresin under reduced pressure. At the end of the distillation, temperature was raised to 180°, and the pressure was reduced to 1 mm.

The yield of turpentine was 22.6%, its characteristics were as follows: density, $d_{4}^{25} = 0.8591$, index of refraction, $n_D^{25} = 1.4663$, specific rotation, $[\alpha]_D^{25} = -44.3$.

A batch of 29 Gm. was fractionated in a Todd column fitted with a Vigreux-type tube 80 cm. long and 1.5 cm. inside diameter. The reflux ratio

was 10 to 1 throughout the distillation. Results of the fractionation are presented in Table I.

Judging from the physical characteristics of the fractions, *P. yunnanensis* turpentine consisted of at least 87% *l*- α -pinene. A pinene nitrosochloride was prepared from fraction 2. The nitrosochloride, recrystallized from chloroform by addition of cold methanol, possessed a melting point of 106–107°. Addition of authentic pinene nitrosochloride did not lower the melting point.

A slight drop in density of fraction 5 suggested the presence of another terpene, but there was not enough material to identify it.

Characteristics of the residue (which was not "oily" and was very fragrant) do not point to any appreciable amounts of sesquiterpenes, but they suggest the presence of a dextrorotatory oxygenated compound.

To sum up, *P. yunnanensis* turpentine contains *l*- α -pinene, 87%, possible *l*- β -pinene (small amounts), and oxygenated terpene compounds 5 or 6%. Apparently there were no sesquiterpenes.

ENTIRE PROJECT SUMMARY

This paper completes the project of systematic investigation of chemical composition of gum turpentines of the species of genus *Pinus*. Turpentines of 66 pines have been investigated or, in some instances, re-examined.

Some of the results of our inquiry are given.

We have found ethyl caprylate in the turpentine of *Pinus edulis*. Previously this ester was positively identified only in fusel oil of grape brandy.

A sesquiterpene, albicaulene, a corresponding sesquiterpene alcohol, albicaulol, and a diterpene, eembrene, were all three found in *Pinus albicaulis* turpentine and named. These are apparently new substances, not reported previously in any plants. Later we found albicaulene and albicaulol in several more pines.

A bicyclic sesquiterpene which was found in *Pinus pinceana* turpentine and which was named maderene is also probably a new sesquiterpene. A diterpene occurring in *P. koraiensis* turpentine which formed maleic anhydride adduct, is probably a newly found hydrocarbon.

Longifolene was reported by us in turpentines of many American and Mexican pines, often in large quantities.

We have found methyl chavicol in gum turpentines of several pines.

Linalool was identified in turpentine of *Pinus tenuifolia*.

We have found that paraffin hydrocarbons are not so rare as ingredients of pine gum turpentines as had been previously supposed, *n*-heptane

was found in turpentine of 8 pines; *n*-undecane in 7

Myrcene was found in turpentine of 5 pines and ocimene in 1. Terpinolene was detected in turpentine of 5 pines.

A bicyclic terpene, Δ^3 -carene, proved to be a rather common terpene in American pines. Some varieties of *Pinus ponderosa* contained more than 50 per cent of this terpene.

Besides contributing to knowledge of terpentine chemistry, information reported in the publications of this series has served as a foundation for studying the distribution, variability, and inheritance of terpenes and associated nonter-

pene compounds of the species of the genus *Pinus*.

REFERENCES

- (1) Clausen, R. T., Contribution to the Flora of New Jersey *Torrysta*, 39, 125 (1939)
- (2) Franchet, A., "Plantarum Sinensium Eclogia Tercia" *J. de Bot.*, 13, 253 (1899)
- (3) Herty, C. H., and Dixon, W. C., *J. Am. Chem. Soc.*, 30, 872 (1908)
- (4) Li, H. L., personal communication
- (5) Little, E. L., Jr., "Check list of native and naturalized trees," U. S. Dept. Agr. Handbook, 41, (1953)
- (6) Martinez, M., "Los Pinos Mexicanos Ediciones Botas," 2nd ed. 1948, p. 175
- (7) Shaw, G. R., "The Pines of Mexico," *Publ. Arnold Arb.*, No. 1 (1909)
- (8) Shaw, G. R., "The Genus *Pinus*," *Publ. Arnold Arb.*, No. 5 (1914)
- (9) Wu, C. L., Master's Thesis, Yale University, College of Forestry (1947)

Concentration of an Alfalfa Growth Factor for *Neurospora sitophila* and Its Use in the Microbiological Assay of Pyridoxine*

By ARTHUR F. NOVAK, MARY LOU JONNARD, and JOSEPH A. LIUZZO†

Dehydrated alfalfa leaf meal contains an unidentified factor(s) necessary for maximum growth of *Neurospora sitophila* in Difco Bacto Pyridoxine Assay Medium. A procedure for one-thousand-fold concentration of this factor was developed which involved extraction of dehydrated alfalfa leaf meal with dilute hydrochloric acid, precipitation of inert material with ethanol, removal of impurities soluble in diethyl ether, chloroform, and benzene, followed by chromatographic adsorption of the active substance on fuller's earth, and then elution with hydrochloric acid. Supplementation of 100 Gm. of Difco Bacto Pyridoxine Assay Medium with approximately 0.5 Gm. of concentrate will improve use of the medium for the microbiological assay of pyridoxine in vitamin products containing alfalfa concentrates.

IN A PREVIOUS PUBLICATION (1) in 1953, it was shown that the addition of alfalfa extracts caused excessive growth of *Neurospora sitophila* in a medium considered complete for the microbiological assay of pyridoxine. This method cannot be employed for accurate pyridoxine assays of vitamin products containing alfalfa concentrates because they contain another factor necessary for maximum growth of the test organism. The nature of this substance was studied.

EXPERIMENTAL

Dehydrated alfalfa leaf meal was used as the source of all fractions discussed in this investigation.

Difco Bacto Pyridoxine Assay Medium (Formula revised July 1954) (2), patterned after that described by Stokes, *et al.* (3), and modified by Barton-Wright (4), was employed because it is highly standardized and results can be duplicated reason-

ably. The fact that this commercial product is deficient in an unidentified factor(s) present in alfalfa, but which is required for maximum growth of the assay organism *Neurospora sitophila* 299 ATCC 9276, is the basis for this investigation. Any significant growth response by this organism above that obtained with the basal medium, and caused by supplementation with alfalfa or its fractionation products, was a verification of the presence of an essential substance in the fraction being tested.

Difco Bacto *Neurospora* Culture Agar (2) was used for maintaining a stock culture of the organism, and for preparing the inoculum. This culture was transferred at fifteen-day intervals and stored under refrigeration at 0°. To prepare an inoculum of *Neurospora sitophila*, a transfer was made forty-eight hours prior to the assaying period.

Pyridoxine stock solution was prepared by dissolving 20 mg. of pyridoxine in 200 ml. of distilled water. This standard solution was discarded after one month, and a new solution prepared. It was stored under refrigeration in an amber bottle to prevent decomposition or inactivation. The concentration of pyridoxine used per assay flask (volume 10 ml.) was 0.1 mg.

To rehydrate the assay medium, 5 Gm. Difco

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Bacto Pyridoxine Assay Medium were dissolved in 100 ml of distilled water and boiled for several minutes. The medium was cooled, and distributed in 50 ml Erlenmeyer flasks. Five milliliters of the assay medium and 1 ml of pyridoxine stock solution were added to each flask. This is referred to as the basal medium. Volume in the control flasks was adjusted to 10 ml by the addition of 4 ml of distilled water. Alfalfa concentrates to be tested were then added to the remaining flasks in concentration equivalent to 0.8 Gm of dehydrated alfalfa leaf meal per flask. Preliminary experiments showed this to be the concentration necessary for maximum stimulation of the organism in the basal medium. This was ascertained from tests made by adding different levels of an aqueous extract of dehydrated alfalfa leaf meal to flasks containing the basal medium. Growth response of *Neurospora sitophila* was measured by comparison of mycelial weights. Results are given in Table I.

TABLE I—GROWTH RESPONSE TO INCREASING CONCENTRATIONS OF ALFALFA

Concn of Alfalfa Gm./Flask	Dry Mycelial Wt. ^a mg./Flask
None	48.0
0.2	54.0
0.5	65.2
0.8	76.2
1.6	76.1

^a Each figure is average of six flasks. Least significant difference at 1% level is 2.9 mg.

Fractions failing to cause increased growth of the organism above the control were retested in higher concentrations.

All test flasks were autoclaved at 121° for fifteen minutes, cooled to room temperature, and inoculated with a culture of *Neurospora sitophila*. Inocula was prepared by transferring one loop of spores from a forty-eight hour culture of the organism into 100 ml sterile 1% saline. One drop of this suspension was added to each flask from a sterilized 1-ml pipet, and the flasks were incubated at 30° for five days. At the termination of the incubation period, the mycelia were removed from the flasks with a stiff wire needle, and pressed dry between paper towels. Each mycelium was then dried to constant weight at 100° and weighed.

All pH adjustments were made with hydrochloric acid and sodium hydroxide.

This general procedure was employed throughout the entire experiment to measure the activity of the various alfalfa fractions.

Chemical and Physical Properties of the Undefined Growth Factor(s).—This phase of the project was planned to obtain facts upon which a reasonable commercial procedure for concentration of the factor could be based. The following alfalfa fractions were made in order to show these properties. Every time a separation was obtained, each fraction was tested for growth promoting activity. Fractions with questionable activity were eliminated.

Dehydrated alfalfa leaf meal was extracted with distilled water in a 1 Gm to 5 ml proportion by

autoclaving the mixture at 121° for thirty minutes. Another portion of leaf meal was extracted with 0.1 N hydrochloric acid by the same method. The mixtures were cooled and filtered through Whatman No. 1 paper. Both extracts were adjusted to pH 4.5 and tested for activity. Residues from these extractions were discarded.

The acid extract, which was found to be more active than the aqueous extract, was adjusted to pH 7.2 and the whole extract was precipitated by the addition of 2 volumes of 95% ethanol. The precipitate was separated by filtration through Whatman No. 1 paper and dissolved in water. Ethanol was removed from the filtrate by boiling. Both the aqueous solution of the precipitate and the filtrate fractions were tested for activity.

The fact that the factor(s) is extracted in acid solution under high temperature is verification of its stability in solution at a low pH. To determine whether or not the factor(s) is stable to heat in alkaline solution, the ethanol filtrate was adjusted to pH 12.0 and boiled for fifteen minutes.

Twenty-milliliter aliquots of the ethanol filtrate were adjusted to pH 4.5 and each extracted by three repeated extractions in a separatory funnel with 30 ml portions of selected solvent. Solvents used for extractions were diethyl ether, chloroform, carbon tetrachloride, and benzene. A 20 ml aliquot of the ethanol filtrate was adjusted to pH 8.5 and extracted with diethyl ether in the same manner. The solvent from each extraction was evaporated to dryness on a steam bath, and 4 ml of distilled water was added to each flask to dissolve the residue remaining in the container.

Twenty-milliliter portions of the ethanol filtrate were adjusted to various pH's and passed through 200 x 18 mm chromatographic columns packed half full with the adsorbant. The adsorbants used were activated carbon (Darco), fuller's earth (Sargent), and levigated alumina (Schaar). Each adsorbant selected was mixed half and half with Celite prior to packing in the column. Columns packed with the adsorbants were wetted with water before the active solutions were passed through. After passing the filtrate through the columns, each was eluted with 0.1 N hydrochloric acid.

The ethanol filtrate at pH 8.5 was precipitated by acetone and this precipitate dissolved in water. Acetone was removed from the filtrate by evaporation on a steam bath, and 4 ml of distilled water was added to the residue.

After the solutions were adjusted to pH 4.5, the solvent extracts, aqueous preparations from the solvent extractions, fractions that passed through columns, and the heat treated ethanol filtrate at pH 12.0 were tested for activity. Results are listed in Table II.

To prepare a concentrate of the active substance, 100 Gm of dehydrated alfalfa leaf meal were extracted with 500 ml of 0.1 N hydrochloric acid by autoclaving for thirty minutes at 121°. This preparation was filtered through Whatman No. 1 paper, and the residue washed with distilled water before being discarded. The filtrate and washings were combined and concentrated to 500 ml by direct heat. After adjusting the concentrated filtrate to pH 7.2, two volumes of 95% ethanol were added, stirred, and allowed to stand overnight. A gelatinous precipitate formed which was removed by

TABLE II.—SOME PROPERTIES OF THE UNIDENTIFIED GROWTH FACTORS

Supplements to Basal Medium, Equivalent to 0.8 Gm. Alfalfa	Dry Mycelial Wt., ^a mg./Flask
None	47.5
Ethanol filtrate of the acid extract	74.0
Heat treated ethanol filtrate, pH 12.0	65.0

Extractions of ethanol filtrate:

	No growth
1. Diethyl ether, pH 4.5	
(a) Extract	87.3
(b) Aqueous phase	
2. Diethyl ether, pH 8.5	
(a) Extract	48.0
(b) Aqueous phase	67.0
3. Chloroform, pH 4.5	
(a) Extract	47.1
(b) Aqueous phase	73.2
4. Carbon tetrachloride, pH 4.5	
(a) Extract	46.4
(b) Aqueous phase	85.1
5. Benzene, pH 4.5	
(a) Extract	47.9
(b) Aqueous phase	85.1
6. Acetone, pH 8.5	
(a) Precipitate	61.5
(b) Filtrate	No growth

Chromatographic columns of:

1. Alumina levitated filtrates	
pH 4.7	70.3
pH 7.0	69.4
pH 9.0	48.1
2. Fuller's earth filtrates	
pH 4.5	62.1
pH 7.0	46.7
pH 8.5	47.4
3. Fuller's earth eluate, pH 8.5	65.0
4. Activated carbon, pH 6.0	61.7

^a Each figure is average of six flasks. Least significant difference at 1% level is 2.6 mg.

filtration, washed with 95% ethanol, and discarded. Ethanol was removed from the filtrate by evaporation on a steam bath, and the volume was adjusted to 500 ml. by addition of distilled water. (One milliliter is equivalent to 0.2 Gm. of dehydrated alfalfa leaf meal.) This solution is referred to as the ethanol filtrate fraction.

Twenty milliliters of the ethanol filtrate was adjusted to pH 4.5 and extracted three times with 30-ml. portions of diethyl ether. The ether extracts were discarded, and the remaining solvent in the aqueous phase was adjusted to 20 ml. by the addition of distilled water. This aliquot was designated as Fraction A.

Fraction A was extracted three times with 30-ml. portions of chloroform at pH 4.5. An emulsion formed, but upon standing separated into two separate phases. The solvent extracts were discarded, and the remaining solvent in the aqueous phase was adjusted to 20 ml. with distilled water, and this was called Fraction B.

Fraction B was extracted three times with 30-ml. portions of benzene at pH 4.5. The solvent extracts were discarded and the remaining solvent present in the aqueous phase was removed by steam

evaporation. Volume of the aqueous phase was adjusted to 20 ml. as above, and this solution was called Fraction C.

Fraction C was adjusted to pH 8.5 and passed through a chromatographic column of fuller's earth. Previously it was found that the active substance is adsorbed on the column at this pH. The column was eluted with 0.1 N hydrochloric acid and the acid eluate was concentrated on a steam bath. This solution was called Fraction D and is the final concentrate.

To evaluate each fraction in terms of increased growth of *Neurospora sitophila* in Difco Bacto Pyridoxine Assay Medium, each fraction was tested by comparison with standard controls. To eliminate the possibility that an insufficient quantity of the active substance was present in the final concentrate, it was also evaluated at double concentration. Activity was measured according to the procedure previously discussed, and the results are presented in Table III. A schematic outline of the fractionation procedure is given in Fig. 1.

TABLE III.—ACTIVITY OF ALFALFA CONCENTRATES ON THE GROWTH OF *Neurospora sitophila*

Additions to Basal Medium, Equivalent to 0.8 Gm. Alfalfa	Dry Mycelial Wt., ^a mg./Flask
None (pyridoxine free)	17.5
None (containing pyridoxine)	48.0
0.1 N HCl Extract	88.5
Ethanol filtrate of the acid extract	76.0
Fraction A	87.0
Fraction B	85.0
Fraction C	71.5
Fraction D	71.9
Fraction D (double concentration)	70.1

^a Each figure is average of six flasks. Least significant difference at 1% level is 2.4 mg.

An aqueous solution of the concentrated material remained stable upon exposure to light seventy-two hours and exhibited some fluorescence when exposed to ultraviolet light.

DISCUSSION

A substance causing increased growth of *Neurospora sitophila* in Difco Bacto Pyridoxine Assay Medium has been concentrated from dehydrated alfalfa leaf meal. Various properties of the factor have been established during the concentration procedure.

It was shown experimentally that the growth factor(s) was extracted to a greater extent with 0.1 N hydrochloric acid than with distilled water. Since the acid extract was concentrated by direct heating and this fraction exhibited growth stimulation, it was established that the factor is stable to heating in acidic solution. The ethanol filtrate also showed growth activity when heated in alkaline solution. Therefore the factor is stable to heating in alkaline solution. Since ethanol was evaporated from the ethanol filtrate by direct heating at approximately neutral pH and this fraction proved to

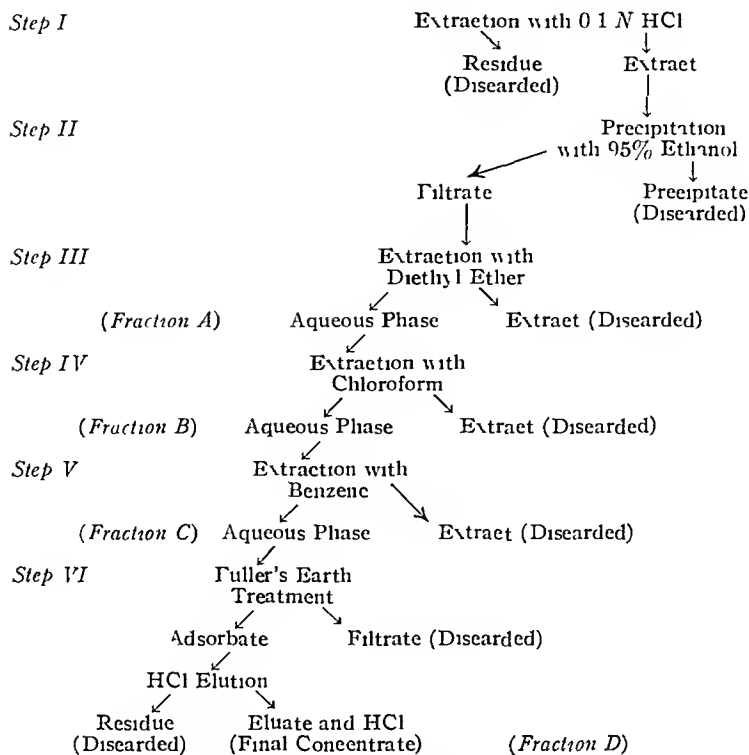


Fig. 1—The scheme of fractionation

be active it was established that the substance is heat stable in neutral solutions

The growth factor is not extracted from the aqueous phase at pH 4.5 in diethyl ether, chloroform, carbon tetrachloride, and benzene, or at pH 8.5 with diethyl ether. It is precipitated from the aqueous phase at pH 8.5 by acetone. It is adsorbed from solution at pH 9.0 by levigated alumina, and at pH 7.0 or 8.5 by fuller's earth, from which it can be eluted with 0.1 N hydrochloric acid. It is not adsorbed from solution at pH 4.7 or 7.0 by levigated alumina, at pH 4.5 by fuller's earth, and at pH 6.0 by activated carbon.

Growth inhibition rather than stimulation resulted when the basal medium was supplemented with either the diethyl ether (pH 4.5) extracted fraction from the ethanol filtrate, or the precipitate formed by the addition of acetone to the ethanol filtrate. This suggests the presence of an inhibitory factor for *Neurospora sitophila*. A growth depression factor was reported in the literature for chicks fed dehydrated alfalfa at high levels in a poultry ration. Lepkovsky, *et al.* (5), found that this factor could be removed from the dehydrated alfalfa by repeated water extraction which suggests that their inhibitory factor and the one described here may be the same. Evidently, a growth depression factor is either destroyed or separated from growth stimulatory factors at this point, because it was not encountered in further separations, or possibly the inhibitory factor is present in such inadequate quantities in the latter fractions that no effect is produced.

The final concentrate prepared in the laboratory

contained a growth promoting factor necessary for maximum growth of *Neurospora sitophila*. This substance is either lacking in the present commercial medium or present in inadequate amounts. Since fraction D caused an increase in growth of 50% above the control as compared to a considerably greater stimulatory effect afforded by the alfalfa hydrolysate, it is apparent that this latter increase in growth is due to at least one additional factor, indicating a separation had been made. Verification was accomplished by failure to increase growth further with Fraction D by increasing the concentration used for the biological test.

The final concentrate represents less than 0.1% of the initial solids. Starting with 100 Gm of alfalfa, the final concentrate weighed approximately 100 mg. It can be stated that 99.9% of the inactive material had been effectively removed from the dehydrated alfalfa leaf meal. Supplementation of 100 Gm of Difco Bacto Pyridoxine Assay Medium with 0.5 Gm of concentrate will improve its use for the microbiological assay of pyridoxine in vitamin products containing physiological concentrates.

REFERENCES

- (1) Novak A F, Gama A R, Iuzzo J A and Rubloff E B. *This Journal* 42, 581 (1953).
- (2) *Difco Manual*, 8th ed. Difco Laboratories, Detroit Mich. 1953, pp. 226-227.
- (3) Stokes J I, Larsen A, Woodward C R Jr, Foster J W. *J Biol Chem* 150, 17 (1943).
- (4) Barton Wright E C. *Analyst* 70, 283 (1915).
- (5) Lepkovsky S, Shaloff W, Peterson D and Perry R. *Poultry Sci* 29, 208 (1949).

The Use of Active Iodine as a Water Disinfectant*

By SHIH L. CHANG

The chemistry of the formation of various species of titrable iodine in solutions of elemental iodine and iodide are described in a quantitative manner. The relative cysticidal efficiencies of various species of titrable iodine and the effect of temperature on cysticidal efficiency of I_2 are described, and equations are given for computing the cysticidal residual I_2 with a given contact time, and vice versa, at varying temperatures, as well as that for computing the cysticidal residual iodine in I_2 - I_2^- systems. The relative viricidal (against Coxsackie B2 virus) efficiencies of various species of titrable iodine and the effect of temperature on viricidal efficiency of I_2 are described, and the employment of the equations used in computing the cysticidal residual iodine for I_2 and I_2 - I_2^- combination, with or without modification, are given. Because of the incompleteness of the data other than those on I_2 at 25°, values obtained in various computations are presented with reservation but are considered as relatively safe for use in practice. The use of preparations of iodine for water disinfection in practice is discussed.

FROM THE AUTHENTIC information on disinfection of water by active halogens (1-7), it is apparent that of the pathogens that may normally find their way into water and water supplies, the cysts of *Endamoeba histolytica* and the enteric viruses, except the adenovirus (7), are more resistant to free chlorine and/or iodine than either the bacteria or cercariae of human schistosomes. Hence, in using active iodine as a water disinfectant, a good margin of safety is provided for the destruction of the latter organisms if the application is based on its effectiveness on the amoebic cysts and one or more of the more resistant enteric viruses.

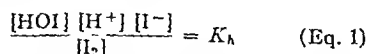
In a quantitative sense, the destruction of microorganisms in water by active iodine is a subject of considerable complexity because of a number of reactions which elemental iodine may undergo in aqueous solutions to form various species of titrable iodine having varying germicidal efficiencies. In two previous communications the writer and his associates have reported on the usefulness of elemental iodine as a water disinfectant (3) and its use for such purpose under field conditions (8). However, in times of emergency, the iodine tablets described (8) may not be available and any of the other preparations, such as tincture of iodine, Lugol's solution, and iodophors, may have to be used, or the water to be treated may have such a high alkalinity as to break the buffering capacity of the dihydrogen pyrophosphate present in the iodine tablets, thus permitting hydrolysis of I_2 , or even further

changes of the hydrolysis product, hypiodous acid. Hence, it is felt that fairly complete information on the use of active iodine as a water disinfectant should be assembled so that it can be used more efficiently under more flexible conditions.

CHEMISTRY OF AQUEOUS SOLUTIONS OF ELEMENTAL IODINE

In various preparations of elemental iodine, iodide is used to improve its stability and solubility, except iodophors, in which the diatomic iodine is stabilized and made more water-soluble by being bound to a nonionic detergent. When a preparation of elemental iodine (excluding iodophors) is dissolved in water, the I_2 may remain in its elemental form, or it may undergo changes involving one or more reactions, depending on the initial concentration of titrable iodine and added iodide, and the pH and temperature of the solution. The formation of iodonines between ammonia or amino compounds and I_2 , fortunately, does not occur under the usual conditions in water disinfection and, therefore, can be omitted from the consideration. The reactions that are of importance are as follows:

Hydrolysis of I_2 —The equilibrium expression for this reaction is:



The value of the equilibrium constant, K_h , is given as 9×10^{-15} at 0° (9), and 3×10^{-13} at 25° (10-12). Since the common I_2 preparations (iodine tablets and tincture of iodine) contain enough iodide to give an I_2 : I^- molar ratio of about 1:1, the percent of titrable iodine existing as I_2 and that as HOI in aqueous solutions of elemental iodine containing 0.5 to 50 p. p. m. of total titrable iodine and enough iodide to give an I_2 : I^- ratio of 1:1.2 have been computed for pH values from 5.0 to 8.0 and are presented graphically in Fig. 1. It should be noted that in the computation the formation of triiodide ion and the decomposition of HOI at pH 8.0 were ignored.

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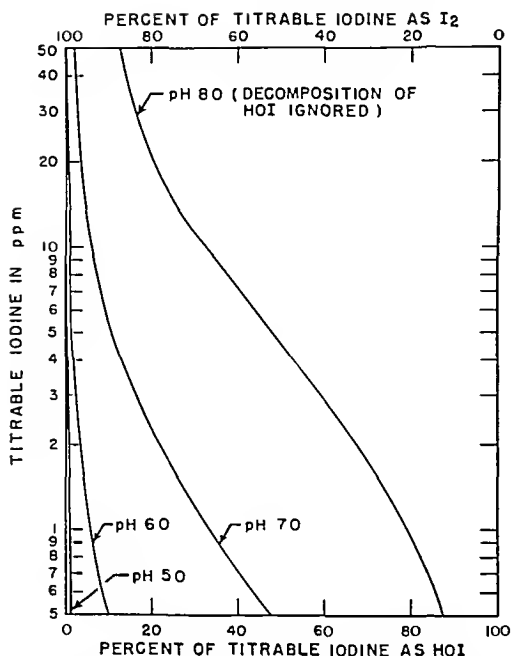


Fig 1—Per cent of titrable iodine as HOI per cent of titrable iodine as I_2 and HOI in aqueous solutions at 25°

In Fig 1 it is seen that in an aqueous solution of elemental iodine containing a total titrable iodine of 0.5 p p m and over an enough iodide to give an I_2 I^- molar ratio of 1:1.2, an insignificant amount of I_2 is hydrolyzed until the pH rises to 6.0 or above. At a total titrable iodine concentration of 5 p p m or greater, an insignificant amount of I_2 is hydrolyzed until the pH rises to 7.0 or above. Hence, for practical purposes, it may be stated that at pH 6.0 or below and total titrable iodine of 0.5 p p m and above, or at pH 7.0 or below and total titrable iodine of 5 p p m and above, all of the titrable iodine exists as I_2 . At pH 7.0 and titrable iodine concentrations below 5 p p m, the amount of I_2 hydrolyzed ranged from about 50% at 0.5 p p m to 10% at 5 p p m. At pH 8.0 the amount of I_2 hydrolyzed by computation ranges from 90% at 0.5 p p m to about 20% at 20–30 p p m, but the slow decomposition of HOI to form iodate upsets the computation to a greater or lesser degree, depending on the buffering system existing and the time of standing.

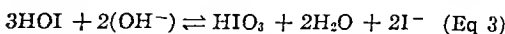
Dissociation of HOI.—At high pH values, 9.0 and over, the HOI formed from hydrolysis of I_2 undergoes dissociation. The equilibrium expression for this reaction is

$$\frac{[H^+][OI^-]}{[HOI]} = K_a \quad (\text{Eq 2})$$

The value of K_a is given as 4.5×10^{-12} at 25° (1). From this value, it is estimated that insignificant amounts of HOI are dissociated until the pH value is raised to over 10.0. Since the HOI decomposes rapidly at pH values of 9.0 and over, it becomes apparent that the existence of OI^- ion is significant

amounts is of such short duration that it has very little, if any, practical importance.

Decomposition of HOI.—At pH values over 8.0, the HOI formed from hydrolysis of I_2 is unstable and decomposes to form iodate and iodide. The reaction or reactions may be briefly expressed as follows:



According to the data of Wyss and Straudskov (12), the decomposition rate in a solution containing 30 p p m or less titrable iodine is slow at pH 8.0 maintained by a borate or carbonate buffer, whereas in the presence of a phosphate buffer, $\frac{2}{3}$ of the HOI is gone in forty minutes. At pH 9.0 the rate of decomposition is so fast that in ten minutes about $\frac{2}{3}$, $\frac{3}{4}$, and $\frac{5}{6}$ of the HOI go into formation of iodate in borate, carbonate, and phosphate buffer, respectively. This rapid decomposition of HOI at high pH values makes it extremely difficult, if not impossible, to compute the amounts of OI^- ion formed in solutions of titrable iodine in which significant amounts of HOI are dissociated.

Formation of I_3^- Ion.—In the presence of added iodide in an aqueous solution, the I_2 goes into formation of tri-iodide ion or periodide. The formation of higher periodide (I_5^- , I_7^- , etc.) has been reported, but does not occur in dilute solutions used in water disinfection work. The equilibrium expression for the formation of tri-iodide ion is as follows

$$\frac{[I_2][I^-]}{[I_3^-]} = K_i \quad (\text{Eq 4})$$

The value of K_i is given as 1.4×10^{-3} at 25° and 0.7×10^{-3} at 0° (13). Since the K_i and temperature have a semilog relationship, the values of K_i at other temperatures can be extrapolated from a curve prepared by plotting these 2 values of K_i against the reciprocal of their respective absolute temperatures on a semilog graph paper. For instance, the values of K_i at 6° and 35° have been found by this method to be 0.82×10^{-3} and 1.85×10^{-3} , respectively (14).

Equation 4 can also be written as

$$[I_3^-] = \frac{[I_2][I^-]}{K_i} \quad (\text{Eq 5})$$

From Equation 5 it is seen that the amount of tri-iodide formed in acidic aqueous solutions of elemental iodine with added iodide increases with the increase in the concentration of iodide and total titrable iodine. For convenience of evaluating cysticidal and virucidal efficiencies of solutions containing both I_2 and I_3^- in the parts that follow, the percentages of these two species of titrable iodine in solutions having varying amounts of elemental iodine and iodide at I_2 I^- molar ratios ranging from 1:1.2 to 1:9.6 have been computed and are presented in Fig 2.

Since, as stated before, the common preparations of elemental iodine, such as the iodine tablets and tinctures of iodine, contain I_2 and iodide at a molar ratio close to 1:1, it may be stated for practical purposes that in water disinfection by iodine, all of the titrable iodine exists as I_2 when its initial concentration is 20 p p m or less and the pH is kept

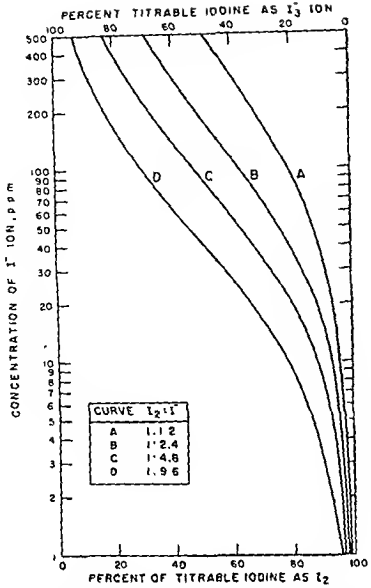


Fig. 2.—Per cent of titrable iodine as I_2 amounts of titrable iodine as I_2 and I_3^- in acidic solutions at 25° and having varying $I_2:I^-$ molar ratios.

under 7.0. As the initial concentration of titrable iodine increases, the amount of I_3^- ion formed is correspondingly increased; but even at a concentration of 500 p. p. m. of titrable iodine, only about 50% of it goes into the formation of I_3^- ion.

CYSTICIDAL EFFICIENCIES OF VARIOUS SPECIES OF TITRABLE IODINE

Elemental Iodine.—At a given temperature and a given concentration of *E. histolytica* cysts, the minimum cysticidal residual I_2 is a function of contact time. The quantitative relationship between the cysticidal residual I_2 and contact time can be expressed by the following equation:

$$C^n t = k \tag{Eq. 6}$$

where C is the residual titrable iodine as I_2 in p. p. m.; t is the contact time in minutes; n is the cysticidal residual I_2 concentration coefficient; and k is a constant, the value of which is dependent on the temperature and the initial cyst concentration.

The cysticidal efficiency of I_2 also increases with the rise in temperature. The quantitative relationship between k and temperature is expressed by the equation:

$$Q_{10} = \left(\frac{k_2}{k_1} \right)^{10/(T_2 - T_1)} \tag{Eq. 7}$$

where Q_{10} is the temperature coefficient for a 10° change within the range where the temperature itself exerts no detrimental effect on cysts, and k_1 and k_2 are constants for temperatures T_1 and T_2 , respectively.

With an initial concentration of 30–60 cysts per ml. of test fluid, which represents on the average a contaminated water containing 0.05–0.1 per cent fecal matter and therefore provides a wide margin

of safety in disinfection work, the relationship between cysticidal I_2 concentrations and contact times has been determined at several temperatures (3, 15, 16). The results observed in these studies are summarized in Fig. 3. From this figure, the cysticidal residual I_2 required for a fixed contact time, or vice versa at any of the presented temperatures can be easily extrapolated. From the slope of, and the spaces separating these four curves, the values for n , k , and Q_{10} have been computed. The value of n was found to be 1.4 and that of Q_{10} , 1.6. The values of k at temperatures ranging from 3 to 44° are presented in Fig. 4. With these two figures and the values of n and Q_{10} , the cysticidal residual I_2 for a fixed contact time, and vice versa, at any temperature besides those shown in Fig. 3 can be conveniently computed.

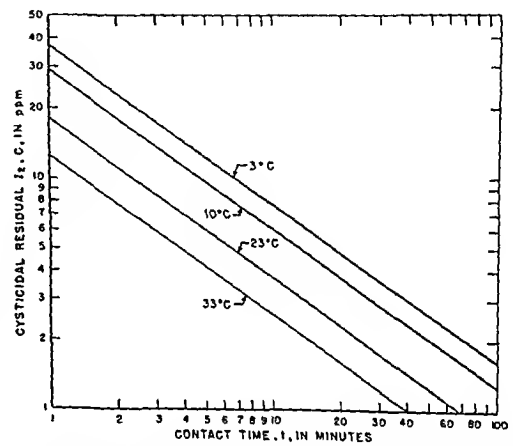


Fig. 3.—Relationship between cysticidal residual titrable iodine as I_2 and contact time at 3° , 10° , 23° , and 35° (at pH below 6.5 and I^- ion concentration under 20 p. p. m.), $n = \text{slope} - 1.4$.

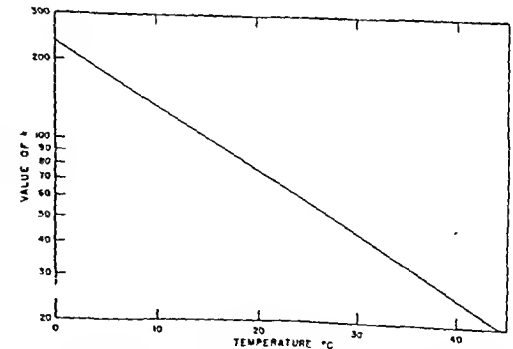


Fig. 4.—Relationship between k and temperature in destruction of amoebic cysts by elemental iodine (I_2).

Hypoiodous Acid.—The cysticidal efficiency of hypoiodous acid (HOI) has been very incompletely ascertained, mainly because of the difficulty encountered in its decomposition at pH values where significant amounts of I_2 would be hydrolyzed.

When the cysticidal efficiency of triiodide was determined (14), an experiment was carried out at pH 8.0 (with a borate buffer) with solutions of elemental iodine and iodide at an $I_2:I^-$ molar ratio of 1:1.2. To minimize the interference caused by decomposition of HOI, a contact time of five minutes was used in the determination. The results obtained in duplicate tests made at 6 and 25° are shown in Table I.

TABLE I.—CYSTICIDAL EFFICIENCY OF SOLUTIONS OF ELEMENTAL IODINE AND IODIDE AT pH 8.0

Temp., °C	Contact Time, in Minutes	Maximum Noncysticidal Residual Iodine, p. p. m. Test 1 Test 2		Minimum Cysticidal Residual Iodine, p. p. m. Test 1 Test 2		Probable Cysticidal Residual Iodine, p. p. m.
6	5	11.9	12.5	13.7	14.5	13.1
25	5	5.8	6.3	6.9	7.2	6.6

The meager amount of information given in Table I does not lend itself to the formulation of a general equation, utilizing the hydrolysis constant of I_2 , pH, and I^- ion concentration to express the relative cysticidal efficiency of HOI to that of I_2 . Nevertheless, it is possible to compute empirically the relative cysticidal efficiencies of these 2 species of titrable iodine. This is done by dividing the difference in p. p. m. between the residual iodine as I_2 alone required to do the same killing and the computed titrable iodine as I_2 in the cysticidal residual at pH 8.0 by the computed p. p. m. of titrable iodine as HOI in the same cysticidal residual. The figure thus obtained is the cysticidal equivalent in p. p. m. of titrable iodine as HOI to 1 p. p. m. of titrable iodine as I_2 . The reciprocal of this cysticidal equivalent of HOI to I_2 is therefore the relative cysticidal efficiency of HOI to that of I_2 . The results obtained in these computations are shown in Table II. In Table II it is seen that the relative cysticidal efficiency of HOI is $1/3$ of that of I_2 at 6°, and is $1/2$ at 25°. It is of interest that these values are a little higher than those obtained by Wyss and Strandskov (12) for bacterial spores.

From the relative cysticidal efficiency of these 2 species of titrable iodine, it is estimated that at 25° and levels of residual iodine below 5.0 p. p. m. the cysticidal titrable iodine at pH 8.0 without decomposition of HOI is about 30% greater than that at pH 7.0 or below, and at levels of residual iodine of and above 5.0 p. p. m. the cysticidal titrable iodine at pH 8.0 is about 20% greater than that at pH 7.0 or below.

Triiodide Ion.—Since the amount of I_3^- ion formed in solutions of elemental iodine and iodide at $I_2:I^-$ molar ratio of about 1:1 becomes more and more significant as the total titrable iodine rises over 20

p. p. m., and since aqueous preparations of elemental iodine and iodide, such as Lugol's solution, may have an $I_2:I^-$ molar ratio as high as 1:3, it is of some importance to know the relative cysticidal efficiency of I_3^- ion to that of I_2 . In a previous report (14) it was shown that the cysticidal efficiency of I_3^- ion is about $1/11$, $1/8$, and $1/7$ that of I_2 at 6°, 25°, and 35° respectively. For determining the cysticidal residual iodine in solutions containing

titrable iodine both as I_2 and I_3^- ion, the following equation was employed:

$$R = A \frac{1 + \frac{[I^-]}{K_1}}{1 + B \frac{[I^-]}{K_1}} \quad (\text{Eq. 8})$$

which was used in an earlier study for determining the relative cysticidal efficiency of OCI^- ion to that of $HOCl$ (2). R represents the total cysticidal residual iodine in p. p. m.; A is the p. p. m. of residual iodine as I_2 alone required to do the same killing; B is the ratio of the cysticidal efficiency of I_3^- ion to that of I_2 ; K_1 is the equilibrium constant of the formation of I_3^- ion; and I^- is the molar concentration of added iodide. With this equation, the values of B at a wide range of temperature have been calculated and are presented in Table III.

TABLE III.—VALUES OF B AT TEMPERATURES FROM 0 TO 48°^a

Temp., °C	Value of B	Temp., °C	Value of B
0	0.077	27	0.127
3	0.083	30	0.132
6	0.088	33	0.138
9	0.094	36	0.144
12	0.099	39	0.149
15	0.105	42	0.154
18	0.110	45	0.160
21	0.116	48	0.166
24	0.121		

^a At temperatures over 18°, the cysticidal efficiency of heat itself is so high that it rapidly kills the cysts.

TABLE II.—CYSTICIDAL EFFICIENCY OF HOI RELATIVE TO THAT OF I_2

Temp., °C	Cysticidal Iodine Residual, p. p. m. at pH 8.0	Computed Titrable Iodine in p. p. m. at pH 8.0 as I_2 HOI		Computed Cysticidal Residual as I_2 Alone, p. p. m.	Cysticidal Residual Equivalent in p. p. m. for HOI I_2	Cysticidal Efficiency of HOI Relative to That of I_2
6	13.1	10.5	2.6	11.3	3.3	0.3
25	6.6	3.9	2.7	5.2	2.1	0.5

With the values of B provided, the cysticidal residual iodine at any temperature between 0 and 48° in a given time with a preparation of elemental iodine having a known $I_2:I^-$ molar ratio can be conveniently computed with Eq. 8.

Iodate Ion.—It has been well known that iodate is nonbactericidal. In a limited number of experiments, it was found that a solution of KIO_3 capable of liberating 10,000 p.p.m. of titrable iodine in the presence of an acid and iodide exhibited no cysticidal effect at 25° and pH 7.0 even after four hours of contact (author's unpublished data).

Iodophors.—Iodophors is a name applied to those titrable iodide compounds that are prepared by dissolving I_2 in nonionic detergents. An acid, usually phosphoric, is added to the preparation to increase its stability and improves its germicidal efficiency. The elemental iodine is supposedly loosely bound to the detergent, thus becoming more soluble in water and less volatile, corrosive, and toxic. Very little information is available on the relative germicidal efficiency of these compounds to that of I_2 . From the extremely limited amount of data obtained with a preparation containing I_2 in polyethylene oxide and urca (17), it appears that the cysticidal efficiency of this compound is comparable to that of solutions of elemental iodine in which a greater portion of the I_2 is converted into I_3^- .

VIRICIDAL EFFICIENCIES OF VARIOUS SPECIES OF TITRABLE IODINE¹

In a very recent study (18) of the viricidal efficiencies of compounds containing chlormelamine and chlormelamine-iodide combination, it was found that of the viruses in the enteric group, the Cocksackie virus (both types A and B) is definitely more resistant to these compounds than either the polio virus or Echo virus. Hence, in another study of the viricidal efficiencies of various species of titrable iodine, the Cocksackie virus (type B1) was employed. As in the preceding study (18), the production and the determination of survival of the virus were made in monkey kidney tissue cultures, and the concentration of virus was estimated by the most probable number method (19). While the current study is still underway, the data gathered thus far have been analyzed, and the results obtained are summarized here.

Elemental Iodine.—From the destruction-rate curves constructed for various concentrations of residual iodine—computed to contain 90-97% I_2 and 3-10% I_3^- at equilibrium—the contact times required for a 99.999% destruction of the virus at 25° were extrapolated and plotted against their respective I_2 residuals on a log-log scale, Fig. 5. Since there was a linear relationship between these 2 variables, Equation 6 was applied for computing the values of n and k , which came out as 0.96 and 58.5, respectively. The fact that the value of n is almost 1.0 indicates that the viricidal residual I_2 and contact time are inversely proportional to each other.

When these data are compared to those obtained for solutions of the iodine tablets used on the Lansing strain of polio virus in a previous study (3), it is noticed that the earlier data were more irregular and that there is some discrepancy in the respective viricidal efficiency findings. This, however, could be readily explained by the fact that the earlier data were obtained with crude mouse brain suspensions used in relatively large amounts in the test water.

The data on the effect of temperature on destruction of the virus by I_2 are incomplete but indicate that the value of Q_{10} may very well be greater than 2.0, which appears to be true also in destruction of the Cocksackie A2 virus by free chlorine (6).

Hypoiodous Acid.—Results on the viricidal efficiency of HOI were irregular in early experiments. As more and more data are being compiled, it appears that HOI is more viricidal than I_2 , based on the fact that the contact times required for a comparable rate of destruction were shorter with solutions of elemental iodine and iodide at an $I_2:I^-$ molar ratio of 1:2 at pH 8.5 (maintained with a borate buffer) than at pH 6.0.

Triiodide Ion.—Repeated experiments made at 25° with solutions of elemental iodine and iodide at very high molar ratios of iodide to I_2 showed that the I_3^- ion is nonviricidal at concentrations as high as 25 p. p. m. of titrable iodine.

Iodate Ion.—In tests made at 25° and pH 7.0 with solutions of KIO_3 containing as much iodate as being capable of liberating 14,500 p. p. m. of titrable iodine in the presence of an acid and iodide, no demonstrable viricidal effect was observed.

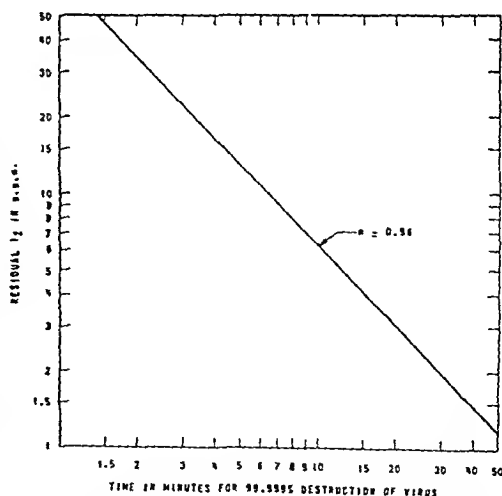


Fig. 5.—Residual I_2 concentration-time relationship in destruction of Cocksackie B1 virus in water at 25° by elemental iodine.

COMPARISON OF THE VIRICIDAL AND CYSTICIDAL EFFICIENCIES OF VARIOUS SPECIES OF TITRABLE IODINE

Elemental Iodine.—It is of importance that while the value of k for the virus destruction by I_2 at 25°

¹ The viricidal data presented in this paper were obtained in a current study carried out by Dr. Gerry Berg, virologist, at the Center.

is very close to that for the cyst destruction (58.5 vs 60.0), the value of n for the former is about $\frac{2}{3}$ of that for the latter (0.96 vs 1.4). Since the negative cultures in cyst survival determinations have been computed to indicate a 99.998% destruction of cysts, the cysticidal and viricidal results are comparable. These values of n and k therefore, indicate that the increase in residual I_2 facilitates a greater cut in cysticidal than in viricidal contact time. For a given residual I_2 , the viricidal contact time in the range not over forty minutes is longer than the cysticidal contact time, and also for a given contact time the viricidal residual I_2 is greater than the cysticidal residual. On the other hand, the increase in viricidal residual I_2 becomes more and more marked over the increase in cysticidal residual as the contact time is reduced toward the one minute period. For instance, when Fig. 5 is compared with the 25 curve in Fig. 3, it is noticed that the corresponding viricidal and cysticidal I_2 residuals are 6.3 and 3.7, 13.6, and 6.0, and 34.0 and 12.5 p.p.m. for the contact times of ten, five, and two minutes, respectively.

Since the value of Q_{10} for cyst destruction by I_2 is 1.6 and that for the virus destruction, greater than 2.0, it becomes apparent that as the temperature is increased above 25° the viricidal efficiency of I_2 increases more and more markedly than the cysticidal efficiency. Assuming that the Q_{10} value for the virus destruction is 2.5, then the viricidal residual I_2 for a ten minute contact at 35° would be 2.5 p.p.m. and that for cyst destruction, 2.2 p.p.m. The contact time for the virus destruction by 5.0 p.p.m. residual I_2 at 35° would be five minutes as against four minutes for the cyst destruction.

Hypoiodous Acid.—The higher viricidal but lower cysticidal efficiency of HOI than I_2 indicates that the rise in pH of the iodine treated water over the 6.0-7.0 range widens the margin of safety in virus destruction but narrows it in cyst destruction. Hence, to provide an adequate treatment of the water, the dosage of iodine should be geared at the cyst destruction and include the amount to compensate for the loss of I_2 to the formation of HOI. Furthermore, being so unstable the HOI cannot be relied on for germicidal activity in long contact times.

Iodide Ion.—The fact that the I_3^- ion at 25° is about $\frac{1}{8}$ as cysticidal as I_2 but has no apparent viricidal activity creates some problem in computing an adequate residual iodine to be maintained in a I_2 - I_3^- system. Since the virus is the more resistant of the two organisms, at least at normal and low temperatures of natural waters, the viricidal data should be used as the basis for computation to give a wider margin of safety in the disinfection process. The zero value of B in Equation 8, therefore, reduces this equation to

$$R = A \left[1 + \frac{[I^-]}{K_1} \right] \quad (\text{Eq. 9})$$

when it is employed for computing the viricidal residual iodine in I_2 - I^- systems.

Iodate Ion.—The lack of demonstrable viricidal and cysticidal effects of iodate ion at high concentrations suffices to consider it nongermicidal and indicates a lack of germicidal activity of decomposition products of HOI at high pH values.

DISCUSSION

An efficient use of halogens and active halogen compounds as water disinfectants demands a clear understanding of their chemical reactions in weak water solutions and the relative germicidal efficiency of each species of active members that may be formed in the solution. In the preceding parts the chemistry of the formation of various species of titrable iodine in water and the cysticidal and viricidal efficiencies of each species formed have been described.

Since natural fresh waters rarely contain iodide, it is felt that given the pH, temperature, and titrable iodine demand of the water, and the iodide content in the preparation of elemental iodine, it should be relatively simple with the information previously presented to compute the dosage of the iodine preparation needed to treat the water adequately for drinking purposes.

If, as appears essential, the pH of the treated water is maintained at levels not over 8.0, only the I_2 , HOI, and I_3^- ion need to be considered in the computation. Since computation involving all of these three species of titrable iodine is too complicated for practical use, and since the amount of HOI formed is relatively insignificant under conditions where a significant amount of I_3^- ion is formed, and vice versa, it is calculated that an additional 10-15% allowance in titrable iodine in computing the adequate residual iodine in I_2 , HOI or I_2 , I_3^- systems should take care of practically all of the more complex conditions.

Since infectious hepatitis has become recently more and more significant as a water borne disease, it appears that basing the computation of the adequate dosage of iodine preparations on the virus destruction not only provides a wider margin of safety for waters of normal temperatures but also is a necessity in areas where this disease occurs in significant numbers. However, when the water temperature is unusually high, say approaching 40°, as may be encountered in the tropics, a slightly wider margin of safety is provided when the computation is based on cyst destruction for iodine preparations containing low (not over 1.2) I_2 - I^- molar ratios. When iodine preparations containing high iodide contents are used for water disinfection, the computation should be based on virus destruction at all temperatures. However, it should be noted that the palatability of the treated water must be taken into consideration in the computation. In treated waters containing more than 10 p.p.m. of residual titrable iodine, odor and taste become more of a problem. Advantage should be taken of the contact time to keep the residual titrable iodine under 10 p.p.m. At pH above the 6.0-7.0 range, the better viricidal efficiency of HOI than I_2 should be ignored.

The recommended dosage (3) of 1 iodine tablet (containing 85 mg of titrable iodine) per qt. for ten minutes at water temperatures prevailing in the warm months in the temperate zone and with iodine demand not over 4.0 p.p.m., or two tablets per qt. for ten minutes at temperatures near 0-5° or at former temperatures but with iodine demand over 4.0 p.p.m., appears to be insufficient for

adequate virus destruction. On the basis of the present information on destruction of the Coxsackie virus, it seems that with either of these applications the contact time should be increased to fifteen minutes for the higher, and twenty minutes for the lower temperatures.

REFERENCES

- (1) Fair, G. M., Chang, S. L., and Morris, J. C., Final report on disinfection of water and related substances to Committee on Medical Research, National Research Council, 1915, p. 50, pp. 216-226, Appendix 74-75, 49, and 87.
- (2) Fair, G. M., Morris, J. C., and Chang, S. L., *J. New Engl. Water Works Assoc.*, 61, 285 (1947).
- (3) Chang, S. L., and Morris, J. C., *Ind. Eng. Chem.*, 45, 1009 (1953).
- (4) Chang, S. L., and Kabler, P. W., *Am. J. Hyg.*, 64, 170 (1956).
- (5) Neefe, J. R., *et al.*, *Am. J. Public Health*, 37, 365 (1947).
- (6) Clarke, N. A., and Kabler, P. W., *Am. J. Hyg.*, 59, 119 (1954).
- (7) Clarke, N. A., Stevenson, R. E., and Kabler, P. W., *ibid.*, 64, 314 (1956).
- (8) Morris, C. J., Chang, S. L., Fair, G. M., and Conant, G. H., Jr., *Ind. Eng. Chem.*, 45, 1013 (1953).

- (9) Jones, G., and Hartman, M. L., *J. Am. Chem. Soc.*, 37, 241 (1915).
- (10) Bray, C. W., *ibid.*, 32, 932 (1910).
- (11) Item, The hydrolysis of iodine and bromine. A Correction *ibid.*, 33, 1485 (1911).
- (12) Wyss, O., and Strandskov, F. B., *Arch. Biochem.*, 6, 261 (1945).
- (13) Jones, G., and Kaplau, B. B., *J. Am. Chem. Soc.*, 50, 1845 (1928).
- (14) Chang, S. L., Baxter, M., and Eisner, L., *Am. J. Hyg.*, 61, 133 (1955).
- (15) Fair, G. M., Chang, S. L., and Morris, J. C., Summary report on germicide data to National Research Council, Committee on Medical Research, for the period from January 1, 1946 to June 30, 1947, pp. 45-47, 1947.
- (16) Item, Annual summary report on germicide to Quartermaster General, U. S. Army for the period from July 1, 1946 to June 30, 1947, pp. 45-47, 1947.
- (17) Fair, G. M., and Chang, S. L., A final report on germicide data to Quartermaster General, U. S. Army for the period from June 23, 1950 to August 31, 1951, p. 93, 1951.
- (18) Chang, S. L., *et al.*, Final report on viricidal efficiency of germicidal rinse formulations to the Office of Quartermaster General, U. S. Army, covering period from September 1955 to October 1956.
- (19) Chang, S. L., *et al.*, Probable Number Method for Viruses by the Tissue Culture Method. Accepted for publication in *Virology*.

Sensitivity to Vaginal Jellies*

Correlation Between Clinical Tests and Animal Tests

By JOHN H. HOLZAEFEL, JOHN S. WARNER, JAY A. BUXTON, and JULIET A. HOWARD

A rabbit vaginal-irritation test described in this article is recommended as a reliable procedure for the evaluation of vaginal jellies and creams. The importance in a drug evaluation test of procedure, type of tissue involved, and method of interpreting the results are discussed.

IN THE DEVELOPMENT of improved contraceptive vaginal jellies, reliable tests for the irritative properties of experimental formulations are required. During the course of our development program, clearance from irritative effects was obtained for an experimental jelly (referred to here as Jelly III) by a Draize (1) rabbit-eye test and a rabbit vagina test involving gross examination of the genitalia and microscopic examination of cross-sections of the vagina. Subsequent clinical field trials revealed that the jelly produced a pronounced irritation which precluded its marketability. Since the previously applied laboratory animal tests were apparently inadequate, a program was initiated to develop a dependable animal mucosal-irritation test which could serve as a preliminary screening method to help avoid the clinical testing of any irritating vaginal preparations.

The approach to the problem involved the design of an animal test which would simulate as closely as possible the experimental procedure and conditions of the clinical vaginal-irritation test. The cytological method which we used in connection with the clinical procedure could also be included in the animal test.

EXPERIMENTAL DESIGN

Rabbits were used as the test animals because they are easy to handle and because their epithelial tissues, in general, are particularly sensitive to irritants. In order to have a fundamental knowledge of the rabbit vaginal tract, anatomic and microscopic studies were carried out. It was noted that the urinary bladder empties into the vagina via the urethral opening 2 to 3 cm. inside the introitus. The lower vagina, below the urethral opening, is somewhat wider and contains many more folds than the upper vagina.

Histological studies made on tissue cross-sections showed that the lining of the lower vagina consists of low cuboidal basal cells and irregular squamous-type surface cells common to the urinary system. The lining of the upper vagina consists of low cuboidal basal cells and columnar surface cells common to the uterus. It was noted that the epithelial

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The microscopic examinations, including the tissue cross-section study and the vaginal smear tests, were made by M. A. Davis, Davidson Laboratories, Columbus, Ohio.

lining is only 3 to 6 cells deep throughout the entire vaginal tract. This tissue should, therefore, be particularly sensitive, and an irritant should cause an immediate reaction because of the close proximity of the irritant to the underlying capillaries, the source of inflammatory exudate.

The rabbit test was first carried out in accordance with the clinical schedule, and a special effort was made to instill the jelly high in the upper vagina above the urethral opening to prevent it from being flushed out during urination. The results of these first tests were very inconsistent and unreliable. It was found that probing the upper vagina of the rabbit triggered urination in many cases. There appeared to be enough back pressure during urination to flush out the entire upper vagina as well as the lower vagina.

Subsequent rabbit tests were carried out by applying the jelly to the lower vagina. It was found that this treatment did not trigger urination. The applications of douche and jelly, the gross observations, and the vaginal smears could be made much more easily and with less mechanical irritation. An additional advantage is the fact that the surface cells of the lower portion of the rabbit vagina are of the same type as those of the human vagina. The objections stated by Carleton and Florey (2) to the use of rabbits in a vaginal irritation test are all avoided by treating only the lower vagina. This modified procedure gave much more reliable results. Reaction to irritating jellies often appeared within twenty-four hours. Three daily applications, with the final observations being made on the fourth day, were found to constitute a very acceptable schedule.

Vaginal smears, made before the first application and one day following the last application, were compared microscopically to determine the degree of irritation indicated by the increase in cellular material. The results were inconsistent and did not correlate well with the results from the gross observations. These shortcomings in the cytological technique can be attributed to both the difficulty in obtaining a valid quantitative smear and the inherently poor quantitative correlation between high level of irritation and available cellular material. The vaginal smear was, therefore, eliminated as a part of the rabbit vaginal irritation test.

METHODS

Clinical Test.—Four patients were used for each jelly tested. The patients were instructed to take a vinegar douche (1/2 cup of white vinegar in 2 quarts of water) followed by the application of the jelly with a vaginal applicator, nightly for six nights. Pelvic examinations to determine gross irritation and vaginal smears to determine cytological response were made before the first application and one or two days following the last application.

Rabbit Test.—Six rabbits, weighing 4 to 6 pounds each, were used for each jelly tested. During the few minutes required for observation and treatment, the animals were strapped to a board with their hind legs spread apart and then turned over on their backs. Gross observations were made by direct visual examination of the external genitalia and by intravaginal examination aided by an otoscope and speculum. Only rabbits that were completely free

of all symptoms of vaginal irritation and sickness were used for the test. A vinegar douche (5 to 10 cc) and then the jelly (10 cc) were applied 1 to 2 cm inside the lower vagina daily for three days. The applications were made with 3 cm blunt tipped soft rubber catheters which were attached to a rubber bulb for the douche and to a 10 cc syringe for the jelly application. An effort was made to fill all of the many folds with jelly as gently as possible.

Gross examinations were made one day following each application. Irritation was classified as erythema, exudate, or edema. Each of these types of irritation was graded as a 1, 2, or 3 degree level, depending upon its severity. Erythema was considered the mildest type of irritation. Each degree of erythema (designated as E) was given an irritation count of 1, 1 e , $E_1 = 1$, $E_2 = 2$, $E_3 = 3$. E_1 was characterized by a slight redness of the mucosa, E_2 was characterized by redness accompanied by occasional distinct blood vessels, and E_3 was characterized by a deep redness accompanied by numerous engorged blood vessels. The presence of an exudate, composed of pus and/or sloughed tissue, was considered more severe than erythema but less severe than edema. Each degree of exudate (designated as X) was given an irritation count of 2, 1 e , $X_1 = 2$, $X_2 = 4$, $X_3 = 6$. X_1 was characterized by a few small globules of pus, X_2 was characterized by large globules of pus, and X_3 was characterized by large amounts of pus and/or sloughed tissue exuding from the vulva. Edema was considered the most severe type of irritation. Each degree of edema (designated as ED) was given an irritation count of 3, 1 e , $ED_1 = 3$, $ED_2 = 6$, $ED_3 = 9$. ED_1 was characterized by a slight swelling of the vulva, ED_2 was characterized by a swelling of the vulva to nearly twice its normal size, and ED_3 was characterized by a swelling of the vulva to several times its normal size. The various irritation counts for the three days were totaled for each rabbit. (For example, a rabbit showing E_1 on the first day, E_2 , X_2 , and ED_1 on the second day, and E_3 , X_3 and ED_3 on the third day would be given irritation counts of 1, 9, and 15 for each of the three days, respectively, making a total of 25.)

The rabbits were allowed to recuperate for at least 10 days before being used for the test again.

Three experimental vaginal jellies were tested clinically and by the rabbit test. Jelly I was prepared as a blank, Jelly II contained 0.10% of an ingredient of unknown irritancy, and Jelly III contained 0.25% of that ingredient.

In the clinical test, Jelly I caused no detectable irritation, the patients reported no undue effects, pelvic examinations revealed no alteration in the vaginal mucous membrane, and the vaginal smears revealed no change in the cytological picture. Jelly II caused moderate irritation, the patients complained of some soreness of the labia, pelvic examinations revealed erythema of the vaginal mucosa, and the vaginal smears revealed an increase in purulent exudate and basal cells. Jelly III caused severe irritation, the patients complained of considerable pain after only a few applications, pelvic examinations revealed erythema, excessive vaginal discharge, and swelling of the labia, and the vaginal smears revealed an increase in purulent exudate and basal cells.

The experimental jellies were tested twice by the rabbit test. The order of irritation, as determined by gross observations (see Table I), was the same as that found by the clinical tests; i. e., I was less irritating than II, and II was less irritating than III. Also, the results from the two series of rabbit tests were found to agree with each other very satisfactorily. Five proprietary vaginal jellies and two proprietary vaginal creams were also tested by the rabbit test to determine what average irritation count would permit a product to be considered acceptable. The results (see Table I) indicate that 5 should be considered a maximum permissible average irritation count. This agrees with the results from the clinical testing of the experimental jellies in which I, having average irritation counts of 1 and 3 in the rabbit test was acceptable and II, having average irritation counts of 8 in the rabbit test, was not acceptable. It should be kept in mind, of course, that the rabbit vaginal-irritation test is intended as a preliminary screening method and not as a substitute for clinical testing.

incomplete understanding of the essentials of the test.

The selection of an appropriate test procedure depends on the use proposed for the drug being evaluated. The most significant results can be expected by the selection of a test that involves the same type of tissue as that to which the drug is to be applied in humans. Occasionally, because of convenience or because of the familiarity with certain testing facilities, a drug is evaluated by a test involving some other type of tissue. In these instances, the method of interpreting the results is particularly important. A striking example is afforded by the clearance that was obtained for the experimental Jelly III by the rabbit-eye test (this test has not been specifically recommended by Draize for evaluating vaginal preparations but has been adopted for this purpose by several testing laboratories). Although Jelly III caused moderate erythema which persisted for two days, the degree of irritation was considered insignificant because the eye returned to normal in three or four days. Subse-

TABLE I.—DATA FROM RABBIT VAGINAL-IRRITATION TEST

Vaginal Preparation	Test Series	Total Irritation Counts per Rabbit ^a						Average
		Rabbit Number						
		1	2	3	4	5	6	
Experimental Jelly I	1	0	0	0	6	0	0	1
	2	8	0	4	2	0	3	3
Experimental Jelly II	1	14	5	9	4	2	12	8
	2	3	17	3	11	9	2	8
Experimental Jelly III	1	29	20	19	11	16	12	18
	2	16	25	19	7	12	17	16
Proprietary Jelly A	1	0	0	1	0	0	0	0
	2	0	3	0	2	0	0	1
Proprietary Jelly B	1	1	2	0	0	0	0	1
	2	0	4	0	0	1	2	1
Proprietary Jelly C	1	2	1	3	3	1	0	2
	2	1	0	0	3	0	0	1
Proprietary Jelly D	1	0	9	0	0	0	5	2
Proprietary Jelly E	1	2	2	0	2	1	2	2
Proprietary Cream A	1	1	0	0	0	1	4	1
	2	0	2	2	2	0	0	1
Proprietary Cream B	1	3	5	3	0	5	13	5
	2	4	3	5	1	8	8	5
	3	13	7	4	5	0	0	5

^a The irritation count is a measure of the various degrees of erythema, exudate, and edema observed on the days following each of the three daily applications of jelly. The counts recorded during the three daily observations were totaled for each rabbit. The highest total irritation count possible is 54.

DISCUSSION

Animal irritation tests involving the skin, eye, penis, or vagina have been used for drug evaluation studies for many years. The rabbit is commonly used as the test animal because it is easy to handle and because its epithelia and mucosa are particularly thin and susceptible to irritants.

The details of procedure in the rabbit vaginal-irritation test described above were found by trial and error to be particularly important. The application of the jelly to the upper vagina instead of to the folds of the lower vagina made the difference between an unreliable test and a reliable test. The misleading information initially obtained from a rabbit vagina test involving gross examination and tissue cross-sectioning probably resulted from an

quent clinical trials showed that such an interpretation was not justified for a vaginal jelly. The usefulness or reliability of the Draize eye test is not disputed in the least. The experience described above emphasizes the importance of establishing a valid basis for interpreting test results. Such a basis in our rabbit vaginal-irritation test was established by correlating animal test results with clinical test results.

Additional tests are in progress to study the effects of composition on the irritancy of vaginal preparations.

REFERENCES

- (1) Draize, J. H., Woodard, G., and Calvery, H. O., *J. Pharmacol. and Exptl. Therap.*, 82, 377 (1944).
- (2) Carleton, H. M., and Florey, H., *J. Obstet. and Gynaecol. Brit. Empire*, 38, 555 (1931).

A Kinetic Study of Acid-Catalyzed Racemization of Epinephrine*

By LOUIS C. SCHROETER and TAKERU HIGUCHI

Racemization of epinephrine follows the first order law with respect to the substrate and appears to be catalyzed by hydrogen ion, the rate increasing very nearly directly with the free ion concentration. The heat of activation of the reaction and the specific rates at several temperatures have been determined. The results suggest that the racemization reaction may prove to be an important route for loss of biological activity of epinephrine solutions at pH values much below pH of 4.

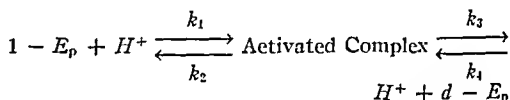
LOSS OF BIOLOGICAL ACTIVITY of aqueous solutions of *l*-epinephrine may occur by either oxidative degradation or through changes in the steric relationship of the asymmetric carbon atom, i. e., racemization. The reactions are essentially independent of one another but occur to varying degrees depending on the solution. The purpose of this work is to present the results from the investigation of the racemization reaction from a chemical kinetic approach.

Although the racemization reaction of epinephrine has been the subject of a number of investigations (1-4), nearly all of these studies have been designed to show the stability of epinephrine under certain conditions and, for the most part, were not concerned with the kinetics of the process or predictions based upon such knowledge. Kisbye (5, 6) has investigated the rate of racemization at higher temperatures and has concluded that the racemization is a monomolecular process. This and our study at low pH values indicate that an entirely different mechanism was responsible for racemization in acid solutions.

The acid catalysis of epinephrine racemization is of such a type to suggest a S_N1 reaction in which water does not stoichiometrically enter into the reaction. This would seem to indicate that the carbonium ion never becomes completely free from the influence of the leaving water molecule. A shielding effect occurs so that the incoming water molecule enters from the opposite side to give a Walden inversion effect: (See mechanism of reaction, page 427.)

Bunton, *et al.* (7), have postulated a similar activated complex for optically active secondary butyl alcohol in perchloric acid solutions. They found the rate of racemization followed Hammett's acidity function, H_0 (8), and that the rate of racemization was always twice the rate of

oxygen exchange. The oxygen exchange was followed by using O^{18} enriched sec. butyl alcohol and H_2O^{18} . The logarithms of the rates of many acid catalyzed reactions have been tabulated and found to conform to $\log k = -H_0 + \text{constant}$ (9). The log of the rate constant for epinephrine racemization has been found to fit this equation. The mechanism may be essentially



where water does not stoichiometrically enter into the equation.

Polarimetric determination of the rate constant involves the measurement of change in optical activity of the *l*-epinephrine solution with respect to time. The physical measurements show the epinephrine solution changing with time from levorotatory ultimately to zero optical activity when equilibrium is established. Considering only the physical measurements involved and the equation

$$1 - E_p \xrightleftharpoons[k_d]{k_c} d - E_p, \text{ where } k_c = k_a$$

the following equation may be derived:

$$a = a_0 e^{-2kt};$$

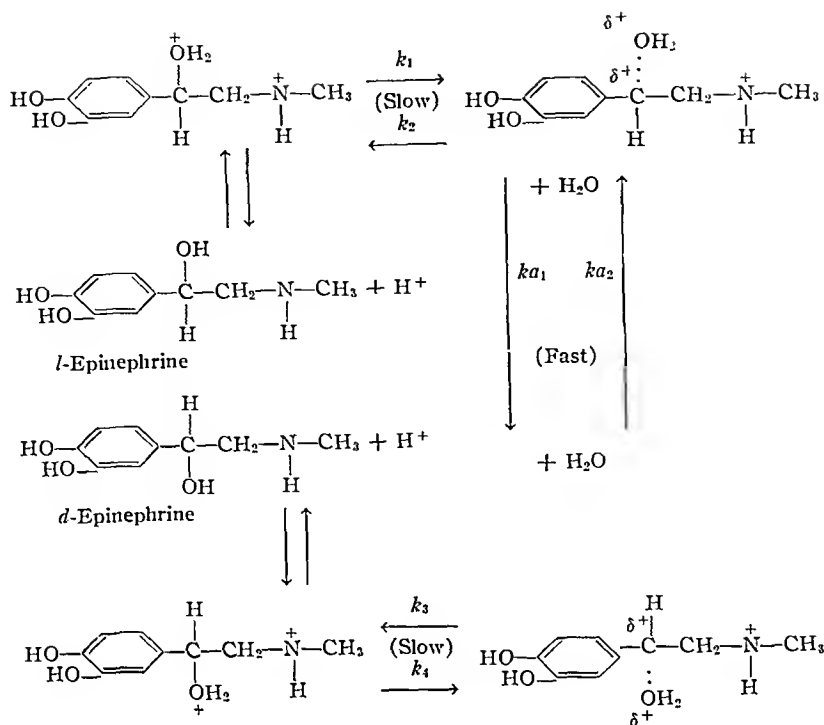
where a is the rotation at any time t and a_0 is the initial rotation, the value $2k$ corresponds to the experimentally determined rate constant. This equation will describe the optical activity of a *l*-epinephrine solution at any given time under isothermal, isohydric conditions.

EXPERIMENTAL

Reagents.—Synthetic *levo*-epinephrine (Parke, Davis & Company), reagent grade perchloric acid, reagent grade hydrochloric acid, reagent grade sodium bisulfite, water redistilled from all-glass apparatus.

* Received August 1957 from School of Pharmacy, University of Wisconsin, Madison.

This study was supported in part by a contract with the Armed Service Medical Procurement Agency, Brooklyn, New York and by a grant from Parke, Davis and Co., Detroit, Mich.



Apparatus.—Zeiss Winkle Polarimeter, constant temperature bath fitted with centrifugal pump and valves, Beckman Model G pH meter with a thermostated cell.

PROCEDURE

Acid Effect and Measurement of pH.—Solutions of epinephrine were prepared by dissolving chemically assayed (10) *l*-epinephrine in standard solutions of perchloric or hydrochloric acid. Perchloric acid was employed because of its single ionization constant and nonvolatility at the experimental temperatures; it was compared with hydrochloric acid at lower temperatures to test the effect of the anion on the racemization and was found to give the same rate of racemization.

Determination of pH of epinephrine in perchloric acid solution was performed at $25^\circ \pm 1^\circ$ on all solutions using the Beckman Model G pH meter calibrated against standard buffers every 0.5 pH unit over the range employed. The pH temperature coefficient of the perchloric acid solutions as determined with the glass-saturated calomel electrode system was found to introduce a small inherent error in this assumption; however, this procedure was found to give more reproducible results than that of making the determinations at the higher temperature of the experiment.

Polarimetric Determinations.—Measurements were made at 589.3 mμ (sodium D line) with the Zeiss Polarimeter fitted with filters and jacketed cells. Temperature was controlled by pumping water from an insulated thermostat with a temperature control of $\pm 0.05^\circ$ through the jacketed polarimeter

cells. Flow of thermostated water through the cells was controlled by valves to limit the initial temperature time lag between cell and thermostat to two minutes and to maintain the cell at the desired temperature within $\pm 0.05^\circ$. Three jacketed cells of various lengths and of different glass composition were employed in the study. The use of different cells served to control vessel effects. The use of the different cells in three determinations of a standard epinephrine solution gave the same rate constant.

The rate of racemization of the thermostated *l*-epinephrine solutions was determined by measuring the change in rotation with respect to time. Typical semi-log plots of optical rotation, α , against time in minutes are shown in Figs. 1, 2, 3, and 4. All of the rotations are corrected to a 2.200-dm. polarimeter tube and represent 0.05 molar epinephrine. The molar rotation, $M[\alpha]_\lambda^T$, may be calculated from any plot by using the expression:

$$M[\alpha]_\lambda^T = \alpha \cdot 1000 / (2.200 \text{ dm.} \times 0.05 \text{ Molar})$$

Effect of Epinephrine Concentration and Bisulfite Concentration.—Effects of the racemization due to oxygen were minimized by using 0.1% sodium bisulfite in several solutions and determining the rate of racemization. The addition of the bisulfite did not significantly change the rate of any of the solutions under the experimental conditions of hydrogen ion concentration and temperature.

The effect of the concentration of epinephrine on the rate of racemization was tested in several determinations by employing 0.05, 0.10, 0.20, and 0.30 molar solutions of epinephrine. In this range

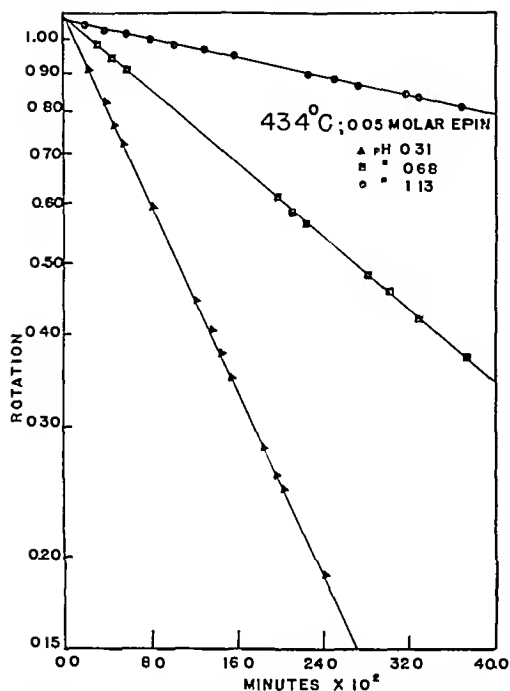


Fig. 1—Semi-log plot of rotation in degrees against time in minutes. Rotations corrected to 2.20-dm tube

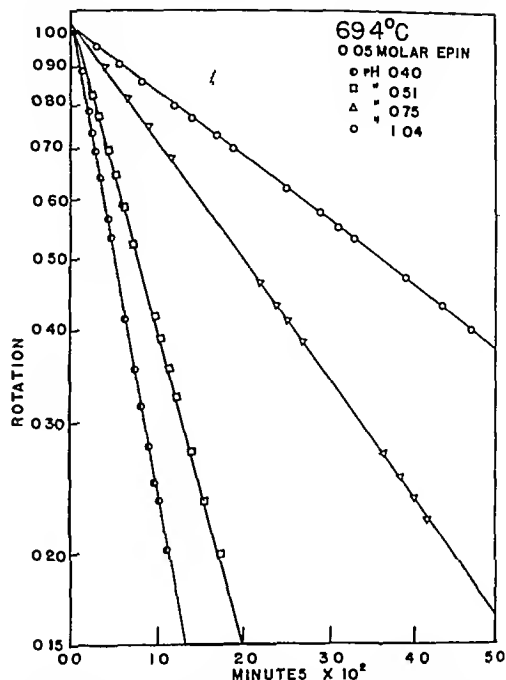


Fig. 3—Semi-log plot of rotation in degrees against time in minutes. Rotations corrected to 2.20-dm tube

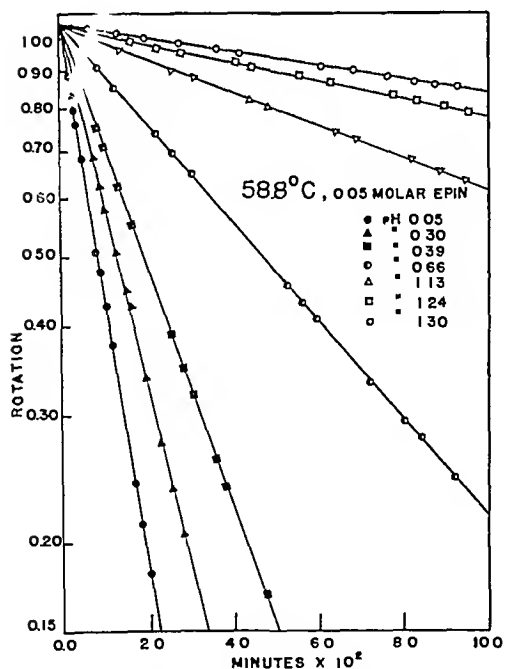


Fig. 2—Semi-log plot of rotation in degrees against time in minutes. Rotations corrected to 2.20-dm. tube.

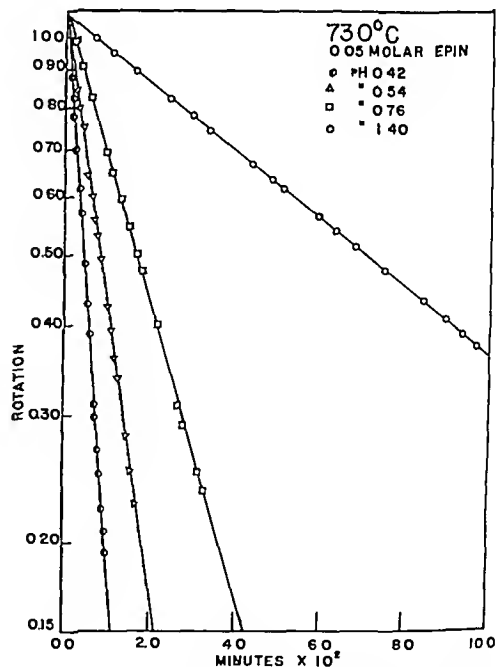


Fig. 4—Semi-log plot of rotation in degrees against time in minutes. Rotations corrected to 2.20-dm. tube.

of concentration the rate appears to be independent of concentration.

RESULTS AND DISCUSSION

The rate of racemization of 0.05 and 0.10 molar epinephrine in perchloric acid and in hydrochloric acid solutions was determined at 43.4°, 53.8°, 69.4°, and 73.0°. Sodium bisulfite in 0.1% concentration was employed in a number of the solutions to test the effect of exclusion of oxygen and also to test the effect of the salt. Figure 5 shows the collected results of some of these determinations in which the logarithm of the rate in reciprocal minutes has been plotted against the pH determined at 25°. The isotherms are essentially parallel and have a negative slope; the value of the rate constant in minutes decreases approximately 6.2×10^{-2} per pH unit increase over the range studied.

Judicious extrapolation of the rate constant to somewhat higher pH values offers a means of prediction of isomer concentration in regions where measurements of the rate change are very difficult due to slowness of the racemization process or where colored degradation products interfere. From the experimental data the following equation was derived:

$$\log K_2 = \log K_1 - [1.21 (\text{pH}_{(2)} - \text{pH}_{(1)})]$$

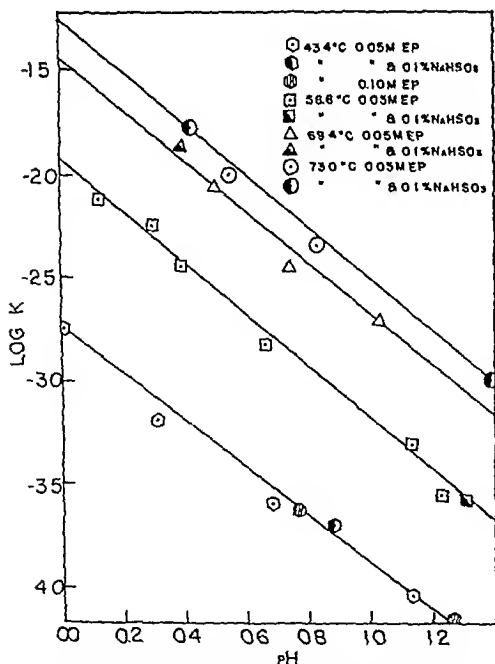


Fig. 5.—Logarithmic plots of isothermal rate constants in reciprocal minutes against pH determined at 25°. Perchloric and hydrochloric acid solutions of epinephrine employed for 43.4° isotherm; perchloric acid employed exclusively in all others. The average slope of the lines is approximately -1.208 .

where K_1 is the experimentally determined rate constant at $\text{pH}_{(1)}$ and pH_2 is greater than pH_1 . From the equation it is possible to predict the rate constant at other isothermal pH values with reasonable accuracy. Determination of several isothermal rate constants will give better results inasmuch as an average value for the rate can be determined and the log of the slope value, -1.21 , can be adjusted to better fit the curve.

Arrhenius' plots of 0.05 and 0.10 molar epinephrine solutions are shown in Fig. 6. The average value for the heat of activation for the process, ΔH_a , as determined from the slopes of the different pH values is 23.19 Kcal mole $^{-1}$; the standard deviation of this value for the six determinations is ± 0.16 Kcal mole $^{-1}$. Plotting the log of the frequency factor, S , from the Arrhenius relationship, $\log k = \log S - \Delta H_a/2.303 RT$, against pH gives Fig. 7. Figure 7 may be used to determine the frequency factor over a moderate range of high hydrogen ion concentration.

Table I compares the experimentally determined half-lives of epinephrine solutions with the predicted values. Calculation of the predicted rates of racemization was made by utilizing Fig. 7 to obtain the log of the frequency factor and the value 23.19 Kcal mole $^{-1}$ for the heat of activation for the process. The same information was utilized to prepare Table II in which the predicted rates and times of maintenance of 95 and 90% levels of optical activity are tabulated. Predicted racemization rates above pH 4.0 should be regarded cautiously since this approaches the limit of reliability for the extrapolated value.

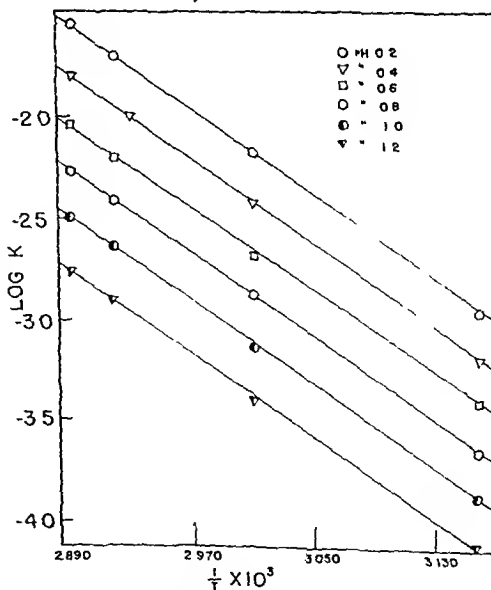


Fig. 6.—Arrhenius' plots for 0.05 and 0.10 molar epinephrine solutions. The logarithm of the rate of racemization, K , is expressed in reciprocal minutes. The average heat of activation, ΔH_a , calculated from the slopes is 23.19 Kcal mole $^{-1}$.

TABLE I—EXPERIMENTAL HALF-LIVES OF EPI-NEPHRINE AT LOW pH COMPARED WITH PREDICTED VALUES

Temperature °C	pH	Half Life in Minutes	
		By Experiment	Predicted ^a
43.4	0.77	2.75×10^3	2.75×10^3
	1.27	9.68×10^4	9.67×10^4
58.8	0.66	4.30×10^2	3.79×10^2
	1.30	2.67×10^3	2.12×10^3
69.4	0.51	7.40×10^1	8.35×10^1
	1.04	3.43×10^2	3.42×10^2
73.0	0.54	6.96×10^1	6.54×10^1
	1.40	6.90×10^2	6.32×10^2

^a Predicted from the logarithmic form of the Arrhenius equation $\log k = \log S - \Delta H / 2.303 RT$ and the half life form for first order equations

CONCLUSIONS

1 The racemization of epinephrine at low pH values is a first order reaction. The rate at low pH values decreases approximately $6.2 \times 10^{-2} \text{ min}^{-1}$ per pH unit increase. The isothermal racemization of epinephrine at different low pH values may be calculated with reasonable accuracy using a simple equation.

2. The average heat of activation for the process determined from Arrhenius' plots is 23.19 Kcal mole⁻¹. This may be used in conjunction with a plot of the Arrhenius frequency factor

TABLE II—PREDICTED RATES AND TIMES OF MAINTENANCE OF A MINIMUM 95 AND 90 PER CENT OPTICAL ACTIVITY

	Predicted Rate Constant Min ⁻¹	Time of Minimum 95% Optical Activity ^a	Time of Minimum 90% Optical Activity ^b
pH 2.5, 25°	2.63×10^{-7}	$1.9 \times 10^5 \text{ min (ca } 4\frac{1}{2} \text{ mo)}$	$4.0 \times 10^5 \text{ min (ca 9 mo)}$
pH 2.5, 35°	9.34×10^{-7}	$5.4 \times 10^4 \text{ min (ca 1 mo)}$	$1.1 \times 10^5 \text{ min (ca 2 mo)}$
pH 3.0, 25°	6.92×10^{-8}	$7.3 \times 10^5 \text{ min (ca 17 mo)}$	$1.5 \times 10^6 \text{ min (ca 35 mo)}$
pH 3.0, 35°	2.45×10^{-7}	$2.0 \times 10^5 \text{ min (ca 5 mo)}$	$4.3 \times 10^5 \text{ min (ca 10 mo)}$
pH 3.5, 25°	1.91×10^{-8}	$2.6 \times 10^6 \text{ min (ca 60 mo)}$	$5.5 \times 10^6 \text{ min (ca 120 mo)}$
pH 3.5, 35°	6.77×10^{-8}	$7.5 \times 10^5 \text{ min (ca 17 mo)}$	$1.6 \times 10^6 \text{ min (ca 36 mo)}$

^a Calculated from the following equation $t_{95\%} = 2.303/k \log (100/95)$

^b Calculated from the following equation $t_{90\%} = 2.303/k \log (100/90)$

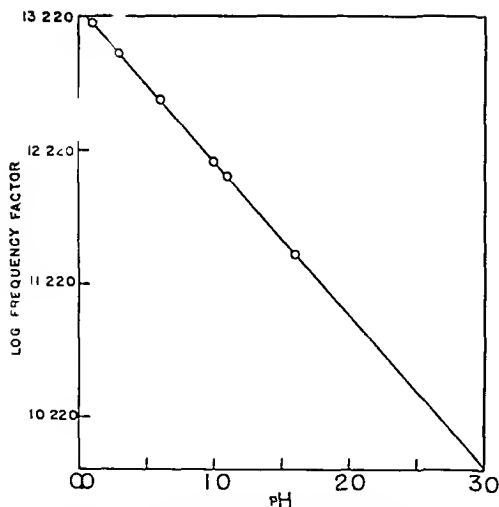


Fig 7—A plot showing pH against the log of the Arrhenius frequency factor, S , where $\log S = \log K + \Delta H / 2.303 RT$

against pH to determine the rate constants at different temperatures and at various low pH values.

3 An equation is described by which the degree of racemization at any time may be calculated if the initial degree of racemization and the rate is known.

REFERENCES

- (1) Berry, H., and West, G. B., *Quart J Pharm and Pharmacol*, 17, 242(1944)
- (2) Hellberg, H., *ibid*, 7, 191(1955)
- (3) Haddock, L. A., *ibid*, 6, 496(1933)
- (4) Florence, G., and Shapira, G., *Arch phys biol*, 17, Suppl., 94(1944)
- (5) Kisbye, J., and Schon, S. A., *Dansk Tidsskr Farm.*, 25, 185(1951)
- (6) Kisbye, J., *Dansk Tidsskr Farm Suppl*, 2, 156(1956)
- (7) Bunton, C. A., Konasiewicz, A., and Llewellyn D. R., *J Chem Soc*, 601(1955)
- (8) Hammett, L. P., "Physical Organic Chemistry," McGraw Hill, New York, 1940
- (9) Hammett, L. P., and Deyrup, A. J., *J Am Chem Soc*, 54, 2721(1932)
- (10) Welsh, L. H., *THIS JOURNAL*, 44, 507(1955)

A Simplified Method to Determine Adrenergic Blocking Activity of Drugs*

By EUGENE N. GREENBLATT† and RICHARD K. THOMS†

A method for determining adrenergic blocking activity utilizing the responses of rabbit isolated thoracic aortic strips to L-epinephrine and L-norepinephrine has been investigated. The abilities of Priscoline, Apresoline, and Benodaine to block L-epinephrine and L-norepinephrine contractions have been determined. In general, the results obtained by this procedure agree with the conclusions reached by other investigators concerning the peripheral actions of these drugs.

FURCHGOTT AND BHADRAKOM have characterized the responses of spirally cut rabbit thoracic aortic strips to such compounds as L-epinephrine, L-norepinephrine, acetylcholine, and nitrates (1).

From their results it appeared that this method might be adapted to the determination of adrenergic blocking activity by observing the effects of drugs to be tested on contractions produced by L-epinephrine and L-norepinephrine. However, because such compounds often have diverse and sometimes antagonistic side actions it was believed necessary to test the method with known sympatholytic and adrenolytic drugs. Because of their varied potencies and side effects Apresoline¹, Priscoline,¹ and Benodaine² were the drugs of choice for this purpose.

EXPERIMENTAL

Methods.—Two spirally cut thoracic aortic strips excised from the same rabbit were mounted in two 30-ml. muscle chambers to record simultaneously on a slow moving kymograph under the conditions recommended by Furchgott and Bhadrakom (1). Thus, experiments were carried out on L-epinephrine and L-norepinephrine concurrently on tissues taken from the same rabbit.

All of the drugs were dissolved in normal saline. The solutions were kept frozen when not in use and kept in an ice bath during the experiment. Even with these precautions, solutions of L-epinephrine and L-norepinephrine had to be prepared weekly. The concentrations of the drugs were adjusted so that all additions to the muscle chambers were made in volumes of 0.2 ml. No temperature effects were noted.

The arterial strips were allowed to attain a state of complete relaxation and then tested for their responses to L-epinephrine and L-arterenol depending on which compound was to be used for the

particular preparation. The concentration of L-epinephrine or L-norepinephrine which produced a marked contraction was the one used as the test dose for that strip.

Throughout the entire investigation these concentrations varied from 10^{-8} (Gm./ml. bath) to 10^{-6} for L-norepinephrine and from 10^{-8} to 10^{-6} for L-epinephrine. The concentrations of the drugs required to block the contractions remained constant in repeated experiments.

After relaxation from the test dose and washing, the drug to be tested for its inhibitory activity was added to the bath followed immediately by L-epinephrine or L-norepinephrine. The reversibility of the blocking actions was determined by adding the test doses of L-epinephrine and L-norepinephrine to the bath, again, after thorough washing. The only exceptions to this general procedure occurred with Priscoline.

Priscoline.—The results of a typical experiment with this drug may be seen in Fig. 1.

It was found that Priscoline itself produced contractions in the arterial strips in concentrations as low as 10^{-7} . However, when Benadryl in a concentration of 6×10^{-7} was added this effect of Priscoline was blocked. It will be noted from Fig. 1 that the shape of the Priscoline contractions differ

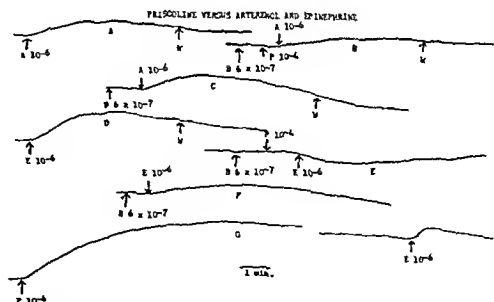


Fig. 1.—A. Shows a normal arterenol (A) contraction. B. In the presence of Benadryl (6×10^{-7}) and Priscoline (10^{-6}) the arterenol contraction is partially inhibited. C. In the presence of Benadryl 6×10^{-7} arterenol exhibits a normal response. D. Shows a normal epinephrine (E) contraction. E. In the presence of Benadryl 6×10^{-7} and Priscoline 10^{-4} , epinephrine elicited a slight reversal. F. In the presence of Benadryl 6×10^{-7} , epinephrine exhibits a normal response. G. In the presence of a stimulating concentration of Priscoline 10^{-6} the epinephrine contraction was not inhibited.

* Received November 15, 1957, from the State University of New York, Brooklyn 3.

Abstracted from a thesis submitted to the Graduate School of the University of Connecticut by Eugene N. Greenblatt in partial fulfillment of the requirements for the degree of Master of Science, and presented to A.P.H.A. Convention, August 1954.

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¹ Generously donated by Ciba Pharmaceutical Products, Inc.

² Generously donated by Merek and Co., Inc.

from those of L-epinephrine and L-norepinephrine being slower and more sustained.

In the presence of Benadryl 6×10^{-7} the test doses of L-epinephrine and L-norepinephrine produced normal responses, but when Priscoline in concentrations as low as 10^{-7} was also added no L-epinephrine or L-norepinephrine contractions could be elicited.

Responses to L-epinephrine and L-norepinephrine were again obtained after washing out the Priscoline and Benadryl.

These results appear to agree with the conclusions of other investigators which state, that in the rabbit, Priscoline is both histaminic and sympatholytic (2-6).

Apresoline.—The results of a typical experiment with this drug may be seen in Figs. 2 and 3.

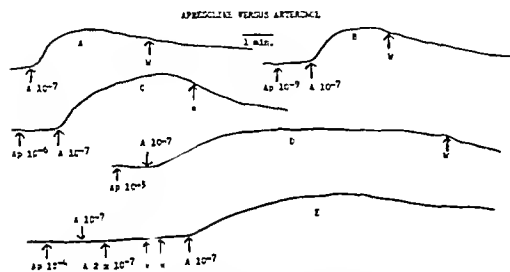


Fig. 2.—A. Shows a normal arterenol (A) contraction. B, C. In concentrations of 10^{-9} through 10^{-8} Apresline (Ap) exhibits no effect on the arterenol contraction. D. Apresoline 10^{-5} partially blocks the arterenol contraction. E. Apresoline 10^{-6} blocks the arterenol contraction, and after washing the arterenol elicits a normal response.

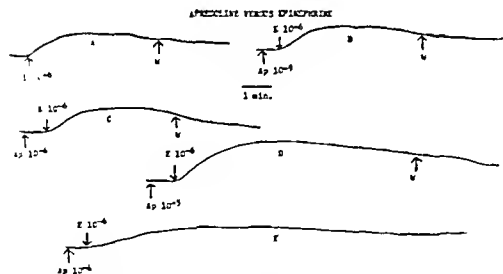


Fig. 3.—A. Shows a normal epinephrine (E) response. B, C. In concentrations of 10^{-9} through 10^{-8} Apresoline (Ap) exhibits no effect on the epinephrine contraction. D. Apresoline 10^{-5} partially blocks the epinephrine contraction. E. Apresoline 10^{-4} elicits a greater inhibition of the epinephrine contraction.

The contractions produced by test doses of L-epinephrine and L-norepinephrine were inhibited by a concentration of 10^{-5} of Apresoline and were completely blocked by a concentration of 10^{-4} . Responses to L-epinephrine and L-norepinephrine were again obtained after washing out the Apresoline.

The fact that these concentrations appear high when compared to those of the other drugs tested appears to agree with the results of other investigators which indicate that Apresoline has a rather low potency with regard to its sympatholytic activity (7, 8).

Benodaine.—The results of a typical experiment with this drug may be seen in Figs. 3, 4, and 5.

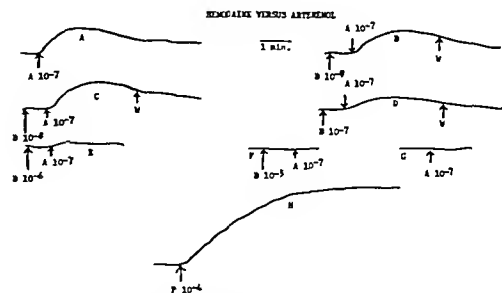


Fig. 4.—A. Shows a normal arterenol (A) contraction. B, C, and D. Benodaine (B) in concentrations of 10^{-9} through 10^{-7} had no effect on arterenol contractions. E. Benodaine 10^{-6} partially blocks the arterenol contraction. F. Benodaine 10^{-5} elicits total block. G. After washing, no further response could be elicited with arterenol. H. Priscoline (P) 10^{-4} elicits a direct muscle stimulation, after apparent irreversible block of the sympathetic receptor sites with Benodaine.

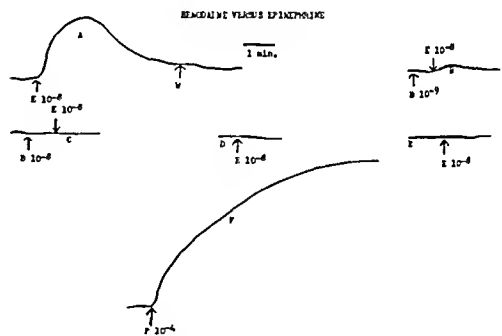


Fig. 5.—A. Shows a normal epinephrine (E) contraction. B. Benodaine (B) 10^{-9} partially blocks the epinephrine contraction. C. Benodaine 10^{-8} totally blocks the epinephrine contraction. D, E. Subsequent addition of epinephrine after washing elicited no response. F. Priscoline (P) 10^{-4} elicits a direct muscle stimulation after apparent irreversible block of the sympathetic receptor sites with Benodaine.

Concentrations of 10^{-9} markedly depressed the contractions produced by epinephrine and concentrations of 10^{-8} blocked them.

Responses to norepinephrine were inhibited by a concentration of 10^{-6} Benodaine and blocked by a concentration of 10^{-5} .

In both cases the blockades were irreversible, in that, after repeated washings the muscle would not respond again to the adrenergic stimulants. However, the muscle did exhibit responses to the histaminic actions of Priscoline

The fact that a much higher concentration of Benodaine is required to influence the response to norepinephrine from that to epinephrine appears to agree with the literature in that Benodaine will inhibit circulating epinephrine, but will have little effect on sympathetic stimulation

REFERENCES

- (1) Furchgott, R. F., and Bhadrakom, S., *J. Pharmacol. Exptl. Therap.*, **108**, 129 (1953)
- (2) Nickerson, M., *Pharmacol. Rev.*, **1**, 27 (1949)
- (3) Chess, D., and Yonkman, F. F., *Proc. Soc. Exptl. Biol. Med.*, **61**, 127 (1946)
- (4) Ahlquist, R. P., et al., *J. Pharmacol. Exptl. Therap.*, **89**, 271 (1947)
- (5) Richards, R. W., *Pharm. J.*, **167**, 31 (1951)
- (6) Fleckenstein, A., *Brit. J. Pharmacol.*, **7**, 31 (1951)
- (7) Gross, F., Druey, J., and Meier, R., *Experientia*, **6**, 19 (1951)
- (8) Britton, J. B., et al., *Fed. Proc.*, **10**, 282 (1951)
- (9) Goldenburg, M., et al., *J. Am. Med. Assoc.*, **135**, 971 (1947)

The Polarographic Behavior of Certain Antioxidants at the Wax-Impregnated Graphite Electrode*

By ROBERT A. NASH,† DONALD M. SKAUEN, and WILLIAM C. PURDY

The anodic polarography of thirty-six phenolic compounds and nine miscellaneous antioxidants at the wax-impregnated graphite electrode is reported. Certain correlations have been made between ring substitution and half-wave potential.

THE ASSESSMENT of antioxidant properties is a matter of considerable importance to the pharmacist. Since polarography is rapid and can be conducted at low concentrations in a wide variety of media, it occurred to us that a polarographic study of a range of compounds having known antioxidant properties might prove extremely useful.

Anodic polarography has been studied far less than the cathodic variety. Although useful in some cases, the dropping mercury electrode itself readily undergoes oxidative attack, so that the stationary or rotating platinum microelectrode is often more useful (1). Gaylor and her co-workers (2-4) have recently introduced the technique of anodic polarography at the wax-impregnated graphite electrode. Lord and Rogers (5) found that the graphite electrode could be used for the anodic polarography of organic compounds. There is, however, limited data

comparing the behavior of the graphite electrode with that of any other well established electrodes.

The purpose of this paper is to present polarographic data for a large number of antioxidants and related compounds. These data will be obtained on the wax-impregnated graphite electrode.

EXPERIMENTAL

Method.—The method of polarography with wax-impregnated graphite electrodes (3) was adapted for use with the Fisher "Electropode." The galvanometer was calibrated in microamperes per scale unit at selected sensitivities by inserting known resistances across the instrument leads and noting the resulting galvanometer displacement as various potentials were applied. For example, readings of 0.0257, 0.0235, and 0.0219 μ A/division were obtained for galvanometer sensitivity settings of 1, 10, and 100X respectively. The potential dial of the instrument was calibrated against a Leeds and Northrup K2 precision potentiometer.

Graphite electrodes¹ fitted to cork stoppers were impregnated with Castorwax² at 100° for a period of two hours. The electrodes were cooled to room temperature and their exposed surfaces were coated with Seal-All³. A length of copper wire, serving as

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† Taken in part from the Ph.D. dissertation of Robert A. Nash, The University of Connecticut, Storrs, Conn.

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¹ Spectroscopic grade Graphite Electrodes of U-1 porosity and 0.25 inch diameter were obtained from the Union Carbon Products Co., Inc., Bay City, Mich.

² Castorwax is a product of the Baker Castor Oil Co., New York, N. Y.

³ Seal All is manufactured by the Allen Products Corporation, Detroit, Mich.

a lead, was fitted to the top of each electrode with a short piece of Tygon tubing and was sealed in place with a small amount of Wood's metal. This eliminated the need for a mercury contact. Fresh surfaces of the electrode were exposed for each run by cutting off lengths of between 0.12 and 0.25 inch from the bottom and abrading the exposed surfaces lightly with fine emery cloth.

Procedure.—Samples of freshly prepared test solution were taken for each run. In the case of water-soluble compounds, 10-millimolar solutions were prepared and diluted 100 times with standard Sorenson pH 7.0 phosphate buffer to obtain working concentrations of 0.1 millimolar. Where water solubility was a problem, the stock solutions were made up in alcohol and diluted with an aqueous alcohol (1:1) buffer to yield apparent pH values of approximately 7.1.

Twenty-milliliter portions of the test solution were placed in a Lingane H-cell fitted with a potassium chloride-agar plug and a saturated calomel electrode (see Fig 1). The cell was placed in a

ing the H-cell. The polarograms were plotted manually.

DISCUSSION OF RESULTS

The polarographic behavior of thirty-six substituted phenols, which have been used as antioxidants, is collected in Table I. These compounds are listed in order of decreasing half-wave potential. Included, also, are the limiting currents (6) for the compounds, measured in a solution at pH 7.0 and containing a 0.1 millimolar concentration of the electroactive material. The α -values (7) for these compounds are also given.

In the case of catechol (8) and gallic acid (9), the results obtained in this study compare favorably with those obtained at a platinum electrode. Although previous studies at the graphite electrode were conducted at pH values other than 7.0, the results obtained here are in line.

The largest single class of antioxidants are the butylated derivatives of phenol. Their insolubility in water and relative lack of toxicity have made them ideally suited for the protection of fats and oils. The half-wave potentials of seven of these butylated phenols, (i.e., "Tetrabutyl Bisphenol A," 2,4-dimethyl-6-*tert*-butylphenol, 2,4,6-tri-*tert*-butylphenol, "Tenox-BHA," 2,6-di-*tert*-butylphenol, "Lederle-2246," and "Tenox-BHT"), have been presented in Table I along with the data on phenol. The half-wave potentials of *m*-*tert*-butylphenol, *o*-*tert*-butylphenol, and *p*-*tert*-butylphenol have been calculated to be 0.520, 0.442, and 0.468 volt vs. SCE at 7.0 (1, 2).

An inspection of these data indicates that butylation in the *ortho*- and *para*- positions has a profound lowering effect on the half-wave potential. *Ortho*-butylation has a greater potential lowering effect than *para*-substitution. Bis structures such as "Tetrabutyl Bisphenol A" and "Lederle 2246" offer no visible advantage in potential lowering to their monophenyl counterparts.

In Table II are found the polarographic data on nine miscellaneous antioxidants and related compounds. Again these data are in line with the work previously reported at the graphite electrode.

In a subsequent paper, correlations will be drawn between the half-wave potential and the antioxygenic efficiency.

SUMMARY

1. Polarography with wax-impregnated graphite electrodes has been adapted to study a wide variety of antioxidants and related compounds. Half-wave potentials, limiting currents, and alpha values are reported for a total of forty-five compounds.

2. Certain correlations between ring substitution and half-wave potential for phenols have been drawn.

3. The method is rapid, can be used at low concentrations in a wide variety of solvents, and should prove extremely useful for the examination of antioxidants.

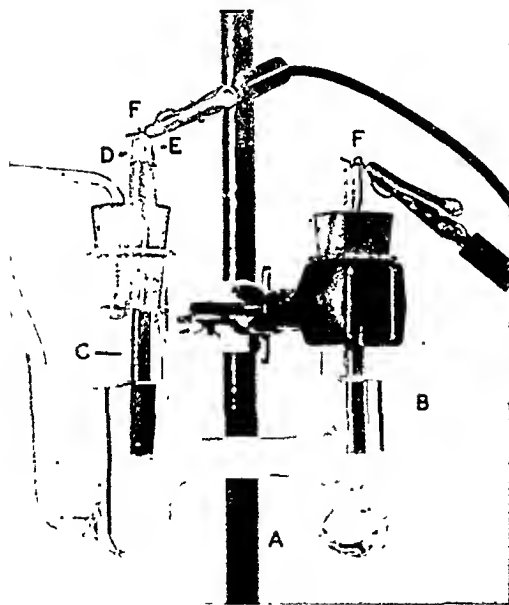


Fig 1—The Lingane H-Cell with a Wax-Impregnated Graphite Electrode. A.—The Lingane H-Cell. B.—The Saturated Calomel Electrode. C.—A Castorwax-Impregnated Graphite Electrode. D.—Tygon tubing. E.—Wood's metal seal. F.—Copper wire lead.

water bath thermostated at $25 \pm 0.5^\circ$. Nitrogen gas was bubbled through the test solutions for fifteen minutes before each run and a nitrogen atmosphere was maintained above the solution during the course of the run. Commercial tank nitrogen was purified by passing the gas through two solutions of hypovanadate and one of test buffer before enter-

TABLE I.—THE POLAROGRAPHIC BEHAVIOR OF CERTAIN SUBSTITUTED PHENOLS

Compound	$E_{1/2}$ in mv vs SCE	i in μ A	α	Solvent System at pH 7.0
Methylparaben ^a	759	1 28	1 46	Aqueous-alcohol
<i>p</i> -Hydroxypropionophenone	745	2 10	0 92	Aqueous-alcohol
<i>p</i> -Nitrophenol	691	3 32	1 21	Aqueous-alcohol
Phenol	537	3 22	0 81	Aqueous
Potassium guaiacol sulfonate	514	1 90	1 10	Aqueous
Vanillin	508	2 64	1 03	Aqueous
Resorcinol	490	3 32	1 05	Aqueous
<i>p</i> -Hydroxyphenylalanine	465	1 15	0 88	Aqueous
Hydroquinone monomethylether	352	2 20	1 25	Aqueous-alcohol
Phloroglucinol	344	2 26	0 87	Aqueous
"Tetrabutyl Bisphenol A" ^b	343	2 06	1 26	Aqueous-alcohol
<i>N</i> -acetyl- <i>p</i> -aminophenol	333	1 71	1 38	Aqueous-alcohol
<i>N</i> - <i>n</i> -butyryl- <i>p</i> -aminophenol	322	1 73	1 49	Aqueous-alcohol
β -Naphthol	310	3 14	1 26	Aqueous-alcohol
2,4,6-Tertiary butylphenol	300	0 86	1 16	Aqueous-alcohol
"Tenox-BHA" ^c	275	1 34	1 34	Aqueous-alcohol
<i>N</i> - <i>n</i> -pelargonyl- <i>p</i> -aminophenol	267	1 81	1 40	Aqueous-alcohol
<i>N</i> - <i>n</i> -stearoyl- <i>p</i> -aminophenol	267	1 93	1 44	Aqueous-alcohol
<i>N</i> - <i>n</i> -lauroyl- <i>p</i> -aminophenol	263	1 40	1 56	Aqueous-alcohol
2,6-Di- <i>tert</i> -butylphenol	208	1 09	1 09	Aqueous-alcohol
"Tenox-BHT" ^d	195	1 02	1 14	Aqueous-alcohol
"Lederle-2246" ^e	168	2 33	1 28	Aqueous-alcohol
α -Naphthol	166	3 39	1 53	Aqueous-alcohol
α -Tocopherol	140	3 50	2 12	Aqueous-alcohol
Catechol	139	1 27	1 77	Aqueous
Ethyl Hydrocaffate	134	1 39	1 81	Aqueous-alcohol
3,4-Dihydroxyphenylalanine	119	2 45	2 03	Aqueous
Ethyl gallate	102	2 13	2 18	Aqueous-alcohol
Propyl gallate	99	2 38	2 12	Aqueous-alcohol
Nordihydroguaiaretic acid	94	1 76	2 39	Aqueous-alcohol
<i>p</i> -Aminophenol hydrochloride	60	1 92	1 15	Aqueous
Gallie acid	59	3 73	1 75	Aqueous
Pyrogallol	33	5 15	1 19	Aqueous
Quercetin	24	6 19	0 69	Aqueous alcohol
Hydroquinone	18	2 24	1 31	Aqueous
Trihydroxybutyrophenone	-10	2 95	1 10	Aqueous-alcohol

^a Methyl *p* hydroxybenzoate, C P, obtained from Heyden Chemical Corporation, New York, N Y^b Obtained from B F Goodrich Chemical Co, C P^c Butylated hydroxyanisole, food grade, obtained from Eastman Chemical Products Inc, Kingsport, Tennessee^d Butylated hydroxytoluene, food grade, obtained from Eastman Chemical Products Inc^e 2,2'-methylenebis (4 methyl 6 tertbutylphenol), C P, obtained from American Cyanamid Co, New York, N Y

TABLE II.—THE POLAROGRAPHIC BEHAVIOR OF SOME MISCELLANEOUS ANTIOXIDANTS AND RELATED COMPOUNDS

Compounds	$E_{1/2}$ in mv vs SCE	i in μ A	α	Solvent system at pH 7.0
Aniline	682	1 46	0 59	Aqueous
2,3-Diphenylindole	552	1 76	1 46	Aqueous-alcohol
Bithionol ^a	457	1 56	0 98	Aqueous alcohol
"Dichlorophene" ^b	452	2 40	0 97	Aqueous-alcohol
Hexachlorophene	406	2 45	1 19	Aqueous-alcohol
Phenothiazene	239	3 13	0 95	Aqueous-alcohol
"Santoquin" ^c	201	1 56	2 67	Aqueous-alcohol
Rutin	199	1 43	3 81	Aqueous-alcohol
<i>N,N'</i> -Diphenyl- <i>p</i> -phenylene diamine	-5	3 10	1 32	Aqueous-alcohol

^a "Aetamer," U S P obtained from Monsanto Chemical Co, St. Louis, Mo^b Obtained from Sindar Corporation, New York, N Y C P^c 6 Ethoxy 2,2,4 trimethyl 1,2 dihydroquinoline, technical obtained from Monsanto Chemical Co

REFERENCES

- (1) Hedenburg, J F and Greiser, H *Anal Chem*, **25**, 1355(1953)
- (2) Gaylor, V I, Elving, P J, and Conrad, A L, *ibid*, **1078**(1953)
- (3) Gaylor, V I, Conrad, A L, and Landerl, J H, *ibid*, **29**, 221(1957)
- (4) *Ibid*, **29**, 228(1957)
- (5) Lord, S S, Jr, and Rogers, L B, *ibid*, **26**, 281(1954)
- (6) Kolthoff, I M, and Lingane, J J, "Polarography," Second ed., Interscience Publishers New York, 1952, p 18
- (7) Evans, P J, and Olson, E C, *J Am Chem Soc*, **79**, 2697(1957)
- (8) Vleck, A K, Mansfield, V, and Krkoskova, D, *Collegium* **24**(1943)
- (9) Vleck, A K, and Spalek, E, *Technická Křesťanská*, **19**, 165(1943)

The Correlation of Polarographic Half-Wave Potentials of Antioxidants with Their Antioxygenic Efficiency*

By ROBERT A. NASH,[†] DONALD M. SKAUEN, and WILLIAM C. PURDY

The anodic polarography of forty-five antioxidants at the wax-impregnated graphite electrode has been presented in a previous paper. In this present communication, certain correlations between polarographic potentials and older potentiometric conventions for the measurement of the potential of an irreversible organic oxidation have been drawn. Furthermore, a correlation between half-wave potential and antioxidant activity is presented. Finally, recommendations are made for the use of ten antioxidants in pharmaceuticals and for eight antioxidants in commercial non-edible preparations.

THERE ARE A NUMBER of ways of quantitatively expressing the potential for an irreversible organic redox reaction. Conant and Pratt (1) introduced the A.O.P. (apparent oxidation potential) in their study of the irreversible oxidation of phenolic compounds. The A.O.P. is defined as the potential developed in thirty minutes by a solution containing the reduced form of the phenol and equimolar amounts of ferrocyanide and ferricyanide. Several years later, Fieser defined the normal oxidation potential (E_0) as the potential difference between a dilute solution of equimolar quantities of the oxidant and the reductant, when neither is ionized, and a hydrogen electrode in the same solvent (2). Subsequently, Fieser (3) defined the critical oxidation potential (E_c) as the potential obtained by an extrapolation of a potential-percentage composition plot for the reaction between the phenol and a suitable reference redox couple.

The polarographic half-wave potential ($E_{1/2}$) for a reversible redox system is equal to the standard oxidation potential minus a constant. This relationship has been shown to apply to the reversible quinone systems (4). For irreversible systems, however, the half-wave potential and the apparent oxidation potential, normal oxidation potential, and critical oxidation potential are only empirical. Since the half-wave potential is much more readily obtainable than the latter three potentials, a study into the relationship between polarographic and potentiometric potentials for systems potentially valuable in pharmaceutical preparations was undertaken.

DISCUSSION

Sixteen compounds were selected to cover a po-

tential range of 1200 millivolts. Half-wave potentials and decomposition potentials (5) were obtained for each compound at the wax-impregnated graphite electrode by the method described in a previous paper (6). These polarographic values were converted to potentials *vs.* N.H.E. by means of the following formulae:

$$E_p = E_{1/2} + 0.059 \text{ pH} - (-0.246) \text{ at } 25^\circ$$

$$E_D = E_d + 0.059 \text{ pH} - (-0.246) \text{ at } 25^\circ$$

In Table I are collected the E_p and E_D potentials together with the corresponding literature values of E_0 and E_c for these sixteen compounds. The E_0 and E_c values reported are for solutions at pH = 7, made up in the same solvents as used in this study. Missing values indicate the lack of literature data for the particular compounds.

An examination of Table I indicates a rather close correlation between E_p and E_0 and between E_D and E_c . The correlation between E_0 and E_c is not nearly so good. The relationship between E_p and E_0 is accepted by all investigators in the field of polarography. The differences between the experimentally determined E_p values and the reported E_0 values can be attributed to one or more of several factors, such as differences in electrode material, ionic strength of solutions, etc. The correlation

TABLE I.—THE OXIDATION POTENTIALS OF SIXTEEN SELECTED COMPOUNDS

Compounds	Potentials in mv. <i>vs.</i> N.H.E.			
	E_0	E_p	E_c	E_D
p-Nitrophenol	..	1350	1433 ^a	1276
Aniline	..	1342	1135	1173
Phenol	1225	1197	1089	1106
Resorcinol	1205	1150	1043	1051
β -Naphthol	..	970	1017	909
Phloroglucinol	..	1004	799	898
α -Naphthol	..	826	797	752
Gallic acid	799	719	..	622
Catechol	793	799	742	749
p-Aminophenol	733	720	673	619
Hydroquinone	710	678	631	634
Pyrogallol	713	683	609	624
α -Tocopherol	770	806	..	761
1,4-Naphthoquinone	484	483	..	519
L-Ascorbic acid	390	300	..	423
9,10-Anthraquinone	154	161	..	223

^a Value calculated by Egloff, *et al.* (7).

* Received October 26, 1957, from the University of Connecticut, Storrs.

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between E_D and E_c could be due to the fact that both these potentials behave like limiting potentials. E_c represents the potential at which a compound first begins its irreversible oxidation of a reversible reference redox system. E_D , on the other hand, is the potential at which a compound first undergoes reduction at an electrode surface.

Although these correlations are not absolute and are subject to such factors as choice of electrode, solvent system, pH, and ionic strength, the results of this study indicate that the half-wave potential, determined at a wax-impregnated graphite electrode, may replace the normal oxidation potential for such compounds as antioxidants. Furthermore, the critical oxidation potential may be replaced by the decomposition potential.

A comparison of the half-wave potential and the antioxygenic efficiency index for twenty-four compounds is presented in Table II. The compounds are arranged in descending half-wave potential order. The antioxygenic efficiency index for each compound was taken from data published by Bickoff (8). The index for each compound was obtained on carotene substrates in mineral oil at 75°.

Table II indicates that the most effective antioxidants are found in the potential region of 30 to 330 millivolts. Based on the results of this table, the

following antioxidants are recommended for use: 1. The "Tenoxs" (butylated hydroxyanisole and butylated hydroxytoluene) for fat soluble pharmaceuticals; 2. Rutin ($E_{1/2} = 199$ mv.), ethyl gallate, propyl gallate ($E_{1/2} = 99$ mv.), ethyl hydrocaffeate, and nordihydroguaiaretic acid for fat soluble pharmaceuticals in which the absence of color is not important; 3. "Santoquin," "Lederle-2246"² ($E_{1/2} = 168$ mv.), phenothiazine ($E_{1/2} = 239$ mv.), 2,4,6-tritert-butylphenol ($E_{1/2} = 300$ mv.), the *N-n*-pelargonyl ($E_{1/2} = 267$ mv.), *N-n*-lauroyl ($E_{1/2} = 263$ mv.), and *N-n*-stearoyl ($E_{1/2} = 267$ mv.) amides of *p*-aminophenol, and 2,6-ditert-butylphenol ($E_{1/2} = 208$ mv.) for commercial, nonedible, fat soluble products; 4. 3,4-Dihydroxyphenylalanine ($E_{1/2} = 119$ mv.), *N*-acetyl ($E_{1/2} = 333$ mv.), and *N-n*-butyryl *p*-aminophenols in aqueous systems on the basis of the correlation established in fat systems.

The present study suggests that anodic polarography should be a simple and rapid general technique for the preliminary assessment of the efficiency index of new potential antioxidants.

Preliminary investigations on certain other antioxidants, chiefly sulfur-containing compounds, were also begun along the lines indicated above. Although qualitative agreement was obtained, the results of these investigations were, however, more difficult to correlate both with the older potentiometric data and with antioxidant efficiency.

SUMMARY

1. Sixteen compounds were employed to establish two relationships between four different oxidation potentials. A correlation between the half-wave potential and the normal oxidation potential and another between the decomposition potential and the critical oxidation potential was demonstrated.

2. A correlation was established between the half-wave potential and the antioxygenic efficiency index for twenty-four of the more important antioxidants. The greatest antioxygenic index for activity is found in the half wave potential region between 30 and 330 millivolts.

3. Recommendations are made for the use of ten antioxidants in pharmaceuticals and for eight antioxidants in commercial nonedible preparations. These compounds are proposed on the basis of the potential-efficiency correlation previously established.

4. The ease with which half-wave potentials can be obtained suggests their use for the prediction of the efficiency index of a newly-synthesized antioxidant.

REFERENCES

- (1) Conant, J. B., and Pratt, M. F., *J. Am. Chem. Soc.*, **48**, 3176(1926).
- (2) Fieser, L. F., *ibid.*, **52**, 4915(1930).
- (3) Fieser, L. F., *ibid.*, **52**, 5201(1930).
- (4) Kolthoff, I. M., and Lingane, J. J., "Polarography,"

TABLE II.—THE CORRELATION BETWEEN ANTIOXYGENIC ACTIVITY AND THE HALF-WAVE POTENTIAL

Compound	$E_{1/2}$ in mv. vs. S.C.E.	Index for Carotene in Mineral Oil at 75°
<i>p</i> -Nitrophenol	691	1
Phenol	537	1
Potassium guaiacol sulfonate	514	6
Vanillin	508	3
<i>p</i> -tert-Butylphenol	468	3
Hydroquinone monomethylether	352	46
<i>N-n</i> -butyryl- <i>p</i> -aminophenol	322	190
β -Naphthol	310	18
2,4-Dimethyl-6-tert-butylphenol	300	188
"Tenox-BHA" ^a	275	130
"Santoquin" ^b	201	112
"Tenox-BHT" ^c	195	142
α -Naphthol	166	154
α -Tocopherol	140	160
Catechol	139	84
Ethyl hydrocaffeate	134	174
Ethyl gallate	102	165
Nordihydroguaiaretic acid	94	208
<i>p</i> -Aminophenol	60	92
Gallie acid	59	7
Pyrogallol	33	155
Hydroquinone	18	8
2,5-Ditert-butylhydroquinone	-558	1
2,5-Diamylhydroquinone	-579	1

^a Butylated hydroxyanisole, food grade, obtained from Eastman Chemical Products, Inc., Kingsport, Tenn.

^b 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, technical, obtained from Monsanto Chemical Co., St. Louis, Mo.

^c Butylated hydroxytoluene, food grade, obtained from Eastman Chemical Products, Inc.

² 2,2-Methylenebis (4-methyl-6-tert-butylphenol), C. P., obtained from American Cyanamid Co., New York, N. Y.

2nd ed., Vol. II, Interscience Publishers, New York, 1952, p. 699.

(5) Kolthoff, I. M., and Lingane, J. J., "Polarography," Second Edition, Vol. I, Interscience Publishers, New York, 1952, Chapt. I.

(6) Nash, R. A., Skauen, D. M., and Purdy, W. C., *THIS JOURNAL*, 47, 433(1958).

(7) Egloff, G., Loury, C. D., Jr., Morrell, J. C., and Dryer, C. G., *Ind. Eng. Chem.*, 25, 804(1933).

(8) Bickoff, E. M., *J. Am. Oil Chem. Soc.*, 28, 65(1951).

The Neuromuscular Activity of a Series of Bis(3-hydroxydimethylpiperidinium Bromide) Ethers*

By A. L. FISHER† and HUGH H. KEASLING

The neuromuscular blocking activity of the bis(3-hydroxydimethylpiperidinium bromide) ethers with 5-8, 10, 12, and 14 methylene groups has been evaluated in the mouse, rat, and hen. The 10, 12, and 14 methylene group derivatives were further tested in dog, rabbit, and frog in an attempt to specify their mode of blockade. Neuromuscular blocking potency increased with increase in number of methylene groups to a maximum at 12. A qualitative change in mode of action occurred with the 12 and 14 methylene compounds. It was concluded that the compounds of this series represent a mixed type of action and that the biological tests utilized do not define the activity as either competitive or depolarizing in character.

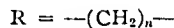
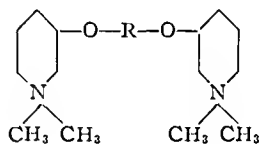
THE OBJECT of this study was an evaluation of the neuromuscular activity, mode of action, and structural relationships to activity of a series of bis(3-hydroxydimethylpiperidinium bromide) ethers. Following quantitative determinations of the neuromuscular potency of the compounds in the mouse, rat, and hen, the qualitative change in mode of action of certain of the compounds was studied further. This report details the results of this investigation.

EXPERIMENTAL

The compounds investigated were the penta-, hexa-, hepta-, octa-, deca-, dodeca-, and tetradecamethylene bis(3-hydroxydimethylpiperidinium bromide) ethers and the bis(3-hydroxypiperidinium bromide) ester of sebacic acid. The structure of these materials is detailed in Fig. 1. All compounds were dissolved in 0.8% saline, and administered as described in each experimental procedure.

Mice.—The technique of Thesleff and Unna (1) was utilized. All compounds were administered by rapid intravenous injection into the tail vein of albino mice. Paralysis was indicated by the in-

ability of the mouse to cling to a screen inclined at 60° from the horizontal. The median paralyzing dose was calculated by the method of probits, using 13 mice at each dose level.



<i>n</i>	Name	Code No.
5	Pentamethylene	JB 361
6	Hexamethylene	JB 367
7	Heptamethylene	JB 407
8	Octamethylene	JB 409
10	Decamethylene	JB 373
12	Dodecamethylene	JB 410
14	Tetradecamethylene	JB 418
$R =$	$-C(=O)-(CH_2)_n-C(=O)-$	JB 392

Fig. 1.—Chemical structure of the bis(3-hydroxydimethylpiperidinium bromide) ethers.

* Received September 30, 1957, from the Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City.

The JB compounds utilized in this study were supplied through the courtesy of H. L. Daiell and H. Friedman. This work was supported in part by a grant from Lakeside Laboratories, Inc., Milwaukee, Wis.

† Smith, Kline & French Fellow in Pharmacology. The material contained in this report is abstracted from a dissertation submitted to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Ph.D.

Rats.—The rat sciatic nerve-gastrocnemius muscle preparation as described by Van Maanen (2) was used with minor modifications. Only male hooded rats weighing from 250 to 400 Gm. were utilized. Each rat received only a single injection of drug.

Chickens.—The sciatic nerve-gastrocnemius muscle of White Leghorn hens weighing between 1.5 and 4.0 Kg. was prepared under pentobarbital

anesthesia (25 mg/Kg) as described by Pelikan, *et al* (3). The tension on the muscle was usually 200 Gm. and held constant in each experiment. Muscle contraction was induced as described by Thesleff and Unna (1), using supramaximal voltages supplied by a Grass stimulator. The contractions were recorded by a counterbalanced lever arm on a smoked drum.

The drug effects were estimated as described by Thesleff and Unna (1). The antagonistic effects of edrophonium on the paralysis was investigated for certain of the compounds of the series by rapid intravenous injection at a dosage of 200 mcg/Kg.

The deca-, dodeca-, and tetradeca-eogeners were investigated further by the following procedures

Dogs.—Dogs were anesthetized with 30 mg./Kg. of pentobarbital sodium. One carotid artery was cannulated and recordings made on a smoked drum via a mercury manometer. The sciatic nerve was isolated and stimulated by supramaximal shocks of 0.2 millisecond duration every ten seconds and the contraction of the anterior tibialis muscle was recorded on the smoked drum. The effects of acetylcholine, histamine, epinephrine, and bilateral carotid occlusion were determined before and after each injection of compound

Rabbits.—Paralysis after intravenous drug administration was measured by loss of the righting response (4). The intravenous LD₅₀ dosage was determined by the method of probits in groups of 13 animals and the therapeutic index was calculated for the deca-, dodeca-, and tetradeca-methylene ethers.

Cholinesterase Studies.—Cholinesterase inhibition was measured by standard manometric techniques using acetyl-β-methyl choline (0.02 M) as substrate and Winthrop-Stearns bovine erythrocyte cholinesterase (20 units/cc). The 50% inhibitory level of the compounds tested was calculated by the method of probits.

Results.—The relative potencies of the compounds in the mouse, rat, and hen are shown in Table I.

TABLE I.—PARALYZING ACTIVITY OF BIS(3-HYDROXYPIPERIDINIUM BROMIDES)

JB No	n	—ED ₅₀ μmoles/Kg—		
		Mouse	Rat	Hen
361	5	2.91	22.50	0.98
367	6	3.34	10.10	1.56
407	7	2.56	7.68	0.43
409	8	1.78	7.28	0.19
373	10	0.26	0.73	0.14
410	12	0.16	0.24	0.10
418	14	0.31	0.38	0.38
392	Sebacia acid ester	159.2	39.80	0.38
<i>d</i> -Tubocurarine		0.08	0.07	0.69
Decamethonium		2.1	7.4	0.015

It will be noted that the dodecamethylene compound (JB 410) is the most potent in all three species and approaches *d*-tubocurarine in potency in the curare susceptible species (rat and mouse) and is

more potent than *d*-tubocurarine in the decamethonium sensitive preparation (hen). The duration of action of these materials was somewhat shorter than that of tubocurarine. The compounds having 5 through 10 methylene groups produced a decamethonium-like contracture in the hen while the dodeca (JB 410) and tetradeca (JB 418) compounds produced a *d*-tubocurarine-like paralysis.

Rabbits.—The action of the deca-, dodeca-, and tetradeca compounds on the loss of righting reflex in the rabbit was examined. As in the other species, the dodecamethylene was the most potent derivative. The intravenous LD₅₀'s for these three compounds were determined and the therapeutic index calculated. These data and a comparison of the therapeutic indices reported for *d*-tubocurarine and Laudolissin under similar experimental conditions are listed in Table II.

TABLE II.—RIGHTING RESPONSE AND TOXICITY IN THE RABBIT

Compound No	(CH ₂) _n	ED ₅₀ mg/Kg	LD ₅₀	Therapeutic Index
JB 373	10	0.160	0.360	2.2
JB 410	12	0.065	0.135	2.1
JB 418	14	0.125	0.240	1.9
<i>d</i> -Tubocurarine	.	.	.	1.8 ^a
Laudolissin ^b	.	.	.	1.7 ^a

^a See reference (5)

^b Decamethylenebis(1,2,3,4-tetrahydro 6,7-dimethoxy 1-1',3',4'-dimethoxy benzyl) 2-methyl isoquinolinium salts

Antagonism.—The antagonistic effect of neostigmine or edrophonium was tested against the deca-, dodeca-, and tetradecamethylene compounds in the hen and dog. In the hen, the decamethylene compound was potentiated, the dodeca not affected, and the tetradeca was weakly antagonized by edrophonium (see Fig. 2). All three compounds were potentiated in the dog by neostigmine.

In mice, the effect of neostigmine was examined against the deca-, dodeca-, and tetradeca-methylene compounds. All were potentiated by neostigmine (see Table III).

TABLE III.—JOINT ACTION OF COMPOUNDS WITH NEOSTIGMINE IN THE MOUSE

JB No	Dosage, mg/Kg	Neostigmine, mg/Kg	No of Mice	Paralyzed, %	Dead, %
373	0.164		26	85	8
373	0.164	0.050	13	100	77
410	0.110		26	80	0
410	0.110	0.025	13	85	23
410	0.110	0.050	13	85	38
410	0.110	0.100	13	100	70
418	0.200		13	54	8
418	0.200	0.100	13	100	77

However, when *d*-tubocurarine chloride and the dodeca-methylene compound were tested together in mice, they had an additive effect as will be noted in Table IV.

TABLE IV.—JOINT ACTION OF *d*-TUBOCURARINE AND JB 410 IN THE MOUSE

Dosage, JB 410	<i>d</i> -Tubo- curarine, mg./Kg.	No. of Mice	Para- lyzed, %	Dead, %
0 100	...	13	15	0
	0.050	13	46	0
0 100	0.050	13	100	100

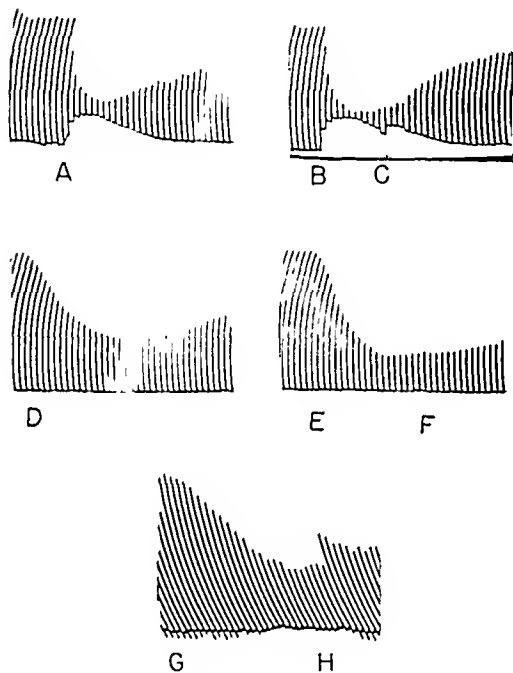


Fig. 2.—Effects of the deca-, dodeca-, and tetradeca-methylene ethers and the effect of edrophonium on the neuromuscular blocking activity of these compounds in the hen sciatic nerve-gastrocnemius muscle preparation. Injections at: A JB 373 100 μ g./Kg.—B JB 373 100 μ g./Kg.—C edrophonium 200 μ g./Kg.—D JB 410 60 μ g./Kg.—E JB 410 60 μ g./Kg.—F edrophonium 200 μ g./Kg.—G JB 418 200 μ g./Kg.—H edrophonium 200 μ g./Kg.

Dog Blood Pressure.—The deca- and dodeca-methylene compounds had no effect upon dog blood pressure up to and including paralyzing doses. Both increased the depressor activity of acetylcholine fourfold which indicated the possibility of cholinesterase inhibition. There was no effect upon the pressor response of epinephrine, the depressor response of histamine, nor upon the pressor response of carotid occlusion.

Frogs.—The action of the deca- and dodeca-methylene compounds was found to be at the neuromuscular junction by use of the Claude Bernard experiment. After injection of these compounds into the ventral lymph sac of frogs, the nonligated leg muscle was unresponsive to electrical stimulation of the sciatic nerve but did respond to direct electrical stimulation. The ligated leg muscle was responsive throughout the experiment to electrical stimulation of the sciatic nerve.

Cholinesterase Inhibition. Manometric studies of the inhibition of true cholinesterases by the deca-, dodeca-, and tetradeca- compounds revealed about $1/100$ the cholinesterase inhibiting activity of neostigmine. The respective molar concentrations for 50% inhibition were decamethylene (JB 373) 4×10^{-6} , dodecamethylene (JB 410) 7.8×10^{-6} , tetradecamethylene (JB 418) 5×10^{-6} and neostigmine 7.9×10^{-8} .

DISCUSSION

Numerous investigations have emphasized the importance of chain length upon neuromuscular potency. While peak neuromuscular blocking activity occurs in the decamethylene member frequently, for example, the methonium series of Barlow and Ing (6) and the isoquinolinium series of Collier (7), Smith, *et al.* (8), found in their series of bis-isoquinoliniums that the hepta- through deca- methylene derivatives were most potent in mice, rabbits, and cats but found the dodecamethylene compound most potent in the frog rectus preparation. Barlow and Ing (6) have shown that the tridecamethylene bis-triethylammonium bromide is the most potent of its series. In the present series the dodecamethylene compound (JB 410) was the most potent in all species tested (see Fig. 3).

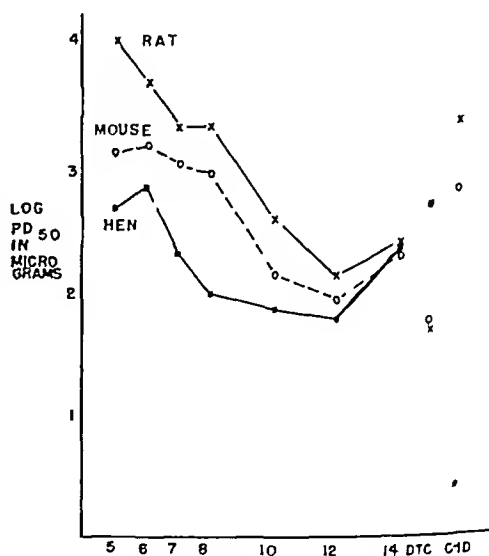


Fig. 3.—Correlation of neuromuscular paralyzing potency with length of the methylene chain.

The change from an ether linkage to an ester linkage markedly reduced the neuromuscular potency. The sebacic acid bis ester of 3 hydroxydimethylpiperidinium bromide (JB 392) was about $1/500$ as active in the mouse and rat as its corresponding ether (JB 373) but was about $1/3$ as active in the hen. However, it provoked contracture in the hen as did JB 373.

Of special interest was the evident change in the mode of action which occurred as the methylene chain was lengthened from the deca- to the dodecamethylene compound. The deca- and shorter chain analogs gave a definite contracture of avian muscle while the dodeca- and tetradeca- did not exhibit this effect. Further, no fasciculations were noted in the rat preparation with the dodeca or tetradeca compounds while the shorter chain members of the series all produced initial fasciculations prior to neuromuscular block. This indication of a change from decamethonium-like to a *d*-tubocurarine-like action was further confirmed by examination of the relative potencies of the deca- and tetradeca-compounds in the hen and rat. The tetradeca- was only $1/2$ as potent as the decamethylene in the hen which is a decamethonium sensitive species, but twice as potent in the rat which is a *d*-tubocurarine sensitive species.

Thesleff and Unna (1) reported no contracture resulted from the penta and hexamethylene members of the methonium series on the hen sciatic nerve-gastrocnemius muscle preparation whereas the higher and lower analogs did produce contracture. However, in this report the lack of contracture producing action was accompanied by a marked decrease in neuromuscular blocking activity. This was evidenced by the fact that the ED_{50} for these noncontracture producing agents was 30-fold that of the next higher analog, the tetramethonium. In our series, such large variations in potency did not occur; in fact the dodeca-, a noncontracture producing compound, was more potent than the most potent contracture provoking drug.

The results of Thesleff and Unna (1) indicated a good correlation between production of contracture in the hen and enhancement of paralysis by neostigmine in the mouse. In this series, this was confirmed by the combination of neostigmine and the decamethylene compound but not with the dodecamethylene or tetradecamethylene compounds; they stimulated no contracture yet were synergized by neostigmine in the mouse.

Zaimis (9) has reported an interesting phenomenon regarding the activity of decamethonium which she regards as evidence for a dual mode of action. She found a decreasing sensitivity of the tibialis muscle of dogs to repeated doses of decamethonium. Further, in association with this tachyphylaxis, the paralyzing effects of decamethonium were antagonized by neostigmine. We were able to confirm her results in this regard with decamethonium. However, the decamethylene compound (JB 373), the most potent drug in this series which appeared to be of the depolarizing type, was tested with the dog tibialis preparation and there was no evidence of tachyphylaxis or antagonism by neostigmine. Further, neither of these effects were noted with the dodecamethylene compound (JB 410), with the tetradecamethylene (JB 418), or with 2,5-bis(*d*-

diethylaminopropylamino)-benzoquinone-bis(benzyl chloride).

At present, mode of neuromuscular blockade is generally divided into two presumptive classes, competitive or tubocurarine-like, and persistent depolarization or decamethonium-like. There is no method which can prove conclusively the exact mechanism of action. Comparison of the end plate potential of the muscle after drug administration is probably the most accurate estimation at the present time for the differentiation of mechanism of action (10). Other techniques have been used, however, which do not require such an elaborate method. Those which indicate a tubocurarine-like activity include the use of tubocurarine sensitive animals such as the rat, antagonism, and reversal of neuromuscular block by the anticholinesterases and; of course, negative responses to the indications of decamethonium-like activity which include potentiation of effect by the anticholinesterases, production of contracture in the hen, and initial fasciculation of muscle prior to the neuromuscular block.

In this series, the penta- through deca-methylene compounds were decamethonium-like in that they were more active in the hen than the rat or mouse, produced initial fasciculations in rat muscle prior to block; and all gave a contracture in the hen. In addition, the decamethylene compound, which was taken as a prototype for the shorter chain members, was potentiated by the anticholinesterases. The dodeca compound was active in both the rat and hen, was decamethonium-like in that it was potentiated by the anticholinesterases and tubocurarine-like in that it produced no initial fasciculations in rat muscle and produced no contracture in the hen. The tetradecamethylene compound appeared tubocurarine-like with respect to the increased sensitivity of rats compared to hens, the lack of contracture provoking properties in the hen while the neuromuscular block was antagonized somewhat by the anticholinesterases. In addition, it produced no fasciculations in rat muscle. However, it was decamethonium-like in regard to its potentiation by the anticholinesterases in mice.

The possibility that the action was other than at the neuromuscular end plate was eliminated for the deca- and dodeca- methylene compounds by the classic Claude Bernard frog experiment. The conclusion must be drawn then, that the compounds of this series represent a mixed type of action and that no single biological test is sufficient in itself to determine the mode of action. An examination of the effects of these mixed type agents on the end plate potentials would be of interest.

REFERENCES

- (1) Thesleff, S. and Unna, K. R., *J. Pharmacol. Exptl. Therap.*, **111**, 99(1954)
- (2) Van Maanen, E. F., *ibid.*, **100**, 362(1950)
- (3) Pelikan, E. W., Smith, C. M., and Unna, K. R., *ibid.*, **111**, 30(1954)
- (4) Collier, H. O. J., Fieller, E. C., and Hall, R. A., *Analyst*, **74**, 583(1910)
- (5) Collier, H. O. J., and Macauley, B., *Brit. J. Pharmacol.*, **7**, 398(1952)
- (6) Barlow, R. B., and Ing, H. R., *ibid.*, **3**, 298(1948)
- (7) Collier, H. O. J., *ibid.*, **7**, 392(1952)
- (8) Smith, C. M., Pelikan, E. W., Maramba, L. R., and Unna, K. R., *J. Pharmacol. Exptl. Therap.*, **108**, 317(1953)
- (9) Zaimis, E., *J. Physiol. (London)*, **122**, 238(1953)
- (10) Burns, B. P., and Paton, W. P. M., *ibid.*, **115**, 1, (1951)

A Report on the Oxidative Degradation of Neutralized Carbopol*

By T. W. SCHWARZ and GERHARD LEVY†

Neutralized Carbopol 934 gels undergo oxidative degradation when exposed to daylight. This reaction is probably catalyzed by trace metals. EDTA, various nonelectrolyte antioxidants and poly-hydroxy compounds may be used to retard this degradation.

AN IMPORTANT REQUIREMENT for pharmaceutical thickening agents is viscosity retention on aging. Carbopol 934, a hydrophilic colloid of high thickening capacity, has been described as an agent of outstanding viscosity stability (1-4). In the course of evaluating Carbopol 934 gels in our laboratory, we have found that this polymer is subject to oxidative degradation. Carbopol 934 gels undergo a marked viscosity decrease at room temperature within two to three weeks if they are exposed to daylight.

In this paper, the stability characteristics of gels made from various salts of Carbopol 934 are reported. Some of these gels were exposed to daylight while others were not. A number of antioxidants, polyhydroxy compounds and a chelating agent were screened for their inhibitory effect on the degradation of the polymer. Viscosity

Fifty milliliter portions of the dispersion were placed in 8 ounce glass jars. The inhibitory agent, dissolved or dispersed in 25 ml of distilled water, was added. A sufficient amount of base in aqueous solution was then added to obtain a pH of 7.0 ± 0.1 . The total solution was brought up to 100 Gm with distilled water.

The jars were stored at room temperature (22 to 25°) in daylight, but protected from direct sunlight. Some of the jars were wrapped in aluminum foil to prevent exposure to light.

The viscosity was measured at 25° with a Brookfield Synchro Lectric Viscometer, Model LVT, spindle No. 4 at 6 r.p.m.

RESULTS AND DISCUSSION

Table I shows the viscosity change of gels made from various salts of Carbopol 934 after 17 days of storage. While the gels which had been protected from light maintained their viscosity, the gels that were exposed to light—regardless of the neutralizing agent used—decreased in viscosity from 33% to 51%.

Table II represents the effect of light on the viscosity of Sodium Carbopol 934 gels in the presence of several antioxidants, polyhydroxy compounds,

TABLE I—THE EFFECT OF DAYLIGHT ON THE VISCOSITY OF 0.7% NEUTRALIZED CARBOPOL 934 GELS

Neutralizing Agent	Initial Viscosity ^a in c.p.s.	Viscosity after 17 days in c.p.s. ^b			
		Exposed to Daylight	Change %	Not Exp. to Daylight	Change %
Sodium hydroxide	57,600	34,100	-41	58,900	+2
Triethanolamine	67,000	45,300	-32	69,200	+3
Ammonium hydroxide	57,200	28,000	-51	59,700	+4
Potassium hydroxide	56,400	37,500	-34	58,600	+4

^a Average of eight samples of the sodium salt of Carbopol 934 and four samples of the other salts.

^b Average of four samples of the sodium salt of Carbopol 934 and two samples of the other salts.

measurements were used for the evaluation of the added chemicals, since the oxidative changes cause a decrease in the viscosity of Carbopol 934 gels.

EXPERIMENTAL

A 1.4% aqueous dispersion of the acid form of Carbopol 934 was prepared in a Waring Blendor

and the sodium salt of ethylenediamine tetracetate acid. All of these agents had some stabilizing effect. In the presence of EDTA no viscosity decrease occurred during storage, which suggests that trace metals act as catalysts in the oxidative degradation of Carbopol 934. The lower initial viscosity of the gels containing EDTA is due to the ionic deswelling which occurs when electrolytes are added to neutralized Carbopol 934 (1, 2). This incompatibility of the polymer with electrolytes prevents the use of ionic antioxidants such as sodium bisulfite, Nordihydroguaric acid, propyl gallate, and ethyl hydroascorbate had to be discarded as antioxidants since these compounds undergo a pronounced color change in the solutions of neutralized Carbopol 934.

* Received October 10, 1957 from the University of California School of Pharmacy, San Francisco 22.

This study was conducted in part under a contract with the Armed Services Medical Procurement Agency.

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* A product of B. I. Goodrich Chemical Company, Cleveland, Ohio.

TABLE II.—THE EFFECT OF DAYLIGHT ON THE VISCOSITY OF 0.7% SODIUM CARBOPOL 934 GELS IN THE PRESENCE OF STABILIZING AGENTS^a

Stabilizing Agent	Concentration, %	Initial Viscosity in c p s	Viscosity, in c p s, after 17 days Exposure to Daylight	Change, %
Butylated Hydroxyanisole ^b	0.05	59,900	53,800	-10
Monothioglycerol ^c	0.5	56,100	54,100	-4
Thiourea	0.5	59,800	56,300	-6
Ethanol 95%	10.0	60,600	49,500	-18
Glycerin	10.0	64,300	55,600	-14
Propylene Glycol	10.0	61,200	53,200	-13
EDTA	0.05 ^d	42,200	42,000	0

^a Average of two samples^b "Sustane," Universal Oil Products Co., Chicago, Ill^c "Thiovanol," Evans Chemetics, Inc., New York, N. Y.^d In terms of the acid form

The results of this study show that pharmaceutical products containing neutralized Carbopol 934 as the thickening agent undergo significant viscosity breakdown when exposed to light. Such products must either be packaged in light-resistant containers or they must contain suitable stabilizing agents.

REFERENCES

- (1) Service Bulletin GC-20, B. F. Goodrich Chemical Company, 1954.
- (2) Cohen, L., *Soap and Chem. Specialties*, 29, No. 11, pp 42 ff., No. 12, pp 50 ff (1956).
- (3) Miscel, B., Powers, J., Ruggiero, J., and Skauen, D., *THIS JOURNAL*, 45, 56 (1956).
- (4) Nobles, W. L., *ibid*, 77, 178 (1955).

The Formation and Structure of the Iron Salt of Benzyl Penicillin Procaine Using Infrared Spectroscopy*

By HERMAN A. SZYMANSKI and NICHOLAS PANZICA

THE FORMATION of coordination compounds may be studied using infrared spectroscopy. Valuable structural details may be obtained by analysis of the absorption bands produced by compound formation. This study was initiated to study the type of bonding and structure which is introduced when the metal iron is coordinated with benzyl penicillin procaine. Since this laboratory was also studying other simple coordination compounds, the results could be interpreted using the analysis suggested by these simple structures.

EXPERIMENTAL

Iron chloride solution was prepared by adding the reagent to anhydrous ethyl ether until a saturated solution was produced. Anhydrous sodium sulfate was added and the solution was mixed well and then centrifuged. Water content was checked using the Karl Fischer method (1). Iron content was

determined by the 1,10 orthophenanthroline method (2). This solution was then stored in a tightly stoppered bottle containing a small amount of anhydrous sodium sulfate.

Benzyl penicillin procaine was obtained from the Charles Pfizer Company, Groton, Conn., and standardized at the Arner Company, Inc., Buffalo, N. Y. Microbiological assay was made using the method suggested in the Federal Register (3).

Benzyl Penicillin Procaine Iron.—This was prepared by several methods, the most successful of which was by dissolving the procaine in chloroform and adding ferric chloride solution described previously. Low temperature increased the yield. Ether was then added to form a precipitate and followed by filtration and washing with ether. Iron content, antibiotic activity relative to the starting material, melting point, and infrared spectra were then determined. The product was then redissolved in methyl alcohol and precipitated again, using ether, and the physical properties rechecked. The best analysis yielded an iron content of 4.3%, m. p. of 140–144°, and antibiotic activity of 105%.

Apparatus.—The infrared spectra were determined on a Baird-Atomic double beam model AB-2 spectrophotometer. Spectra were run in potassium

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bromide pellets, as mulls, and in several solvents.

Discussion of results.—The iron salt of benzyl penicillin sodium has been prepared and analyzed by Jadassohn (4). The iron analysis suggested the possibility of three moles of penicillin coordinating with one mole of iron. Calculations for the compound prepared in this work indicated that an iron content of 4.27% would be the theoretical per cent. This compares favorably with the 4.30% obtained for this compound.

The absorption at 5.6μ for penicillin compounds has been correlated with antibiotic activity (5), a strong absorption indicating large activity. Figure 1 is the spectrum of the coordination compound and the high absorption at 5.6μ agrees with the strong antibiotic activity of this compound.

The analysis of this spectrum has suggested that the peaks at 5.93, 7.87, and 8.50 indicate the procaine portion of the molecule and may be compared

band shifts on coordination compound formation, suggesting the iron may be forming an iron penicillinate.

The doublet at 4.0μ in the coordination compound has been under intensive study by this group since we have obtained this same band with tertiary amine compounds of arsenic and antimony trihalides. The same doublet appears in procaine hydrochloride and amine hydrochloride salts which have been determined in this laboratory. These results therefore suggest that the iron has produced a linkage of the type $(R-NH_2)_3-Fe$ in this compound as a secondary linkage. The primary linkage being a bond between the iron and the acid group in the penicillin forming a penicillinate. Scale models of this proposed structure indicate the geometry would be satisfied by this structure.

Bands at 6.0 and 6.6 generally indicate monosubstituted amides while disubstituted amides show

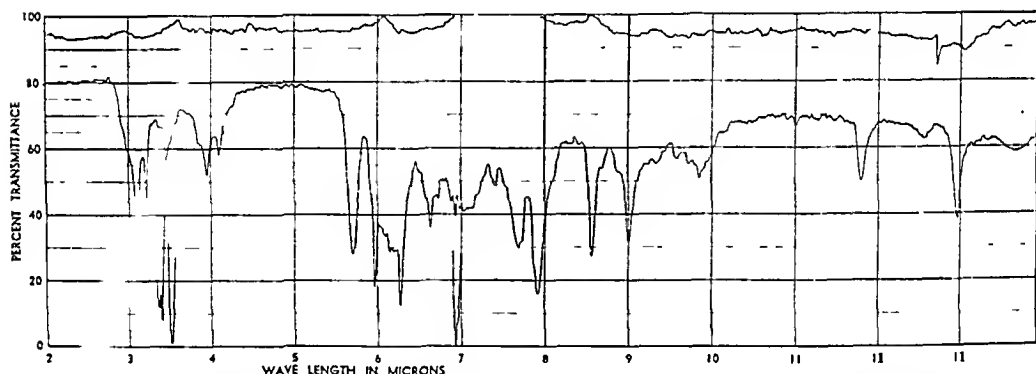


Fig. 1.—Infrared spectrum of the iron salt of benzyl penicillin procaine in potassium bromide.

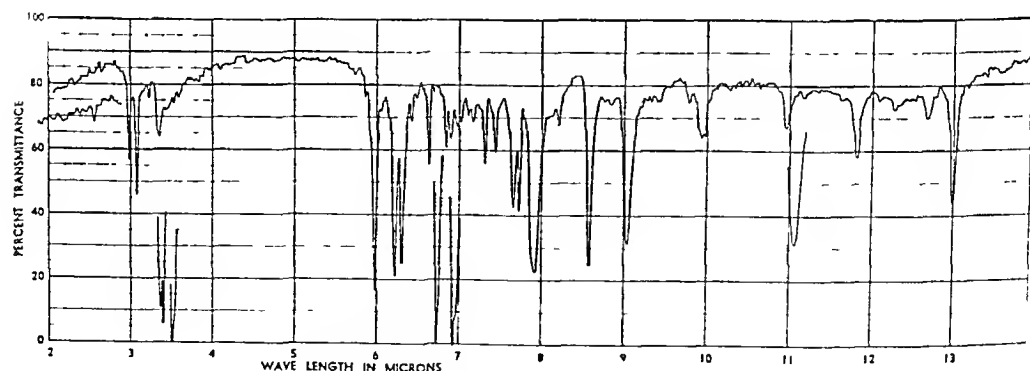


Fig. 2.—Infrared spectrum of procaine hydrochloride in potassium bromide.

to those obtained for procaine hydrochloride and benzyl penicillin procaine (Figs. 2 and 3).

The 6.2μ band in Fig. 3 is probably made up of the carboxylate $C=O$, phenyl group absorption and an absorption due to the procaine molecule. This

bands only at 6.0. In benzyl penicillin procaine as well as its iron compound, no 6.6 band occurs although most penicillins are monosubstituted amides. It is suggested that this band is missing due to hydrogen bond formation between the N-H normally

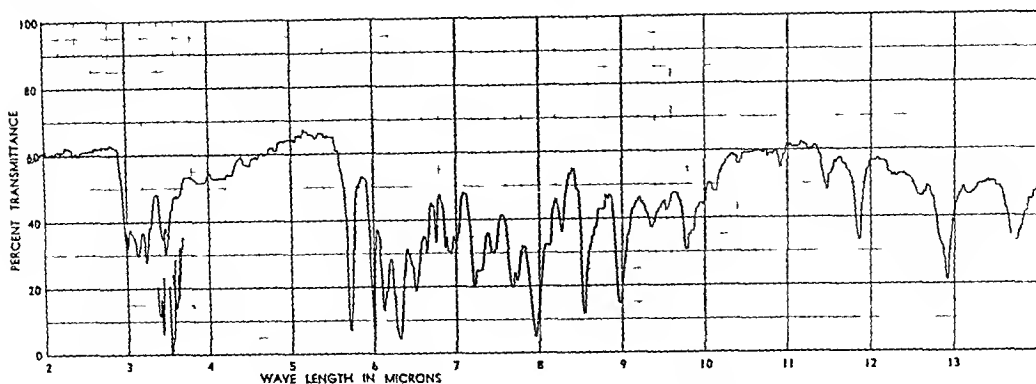


Fig 3 —Infrared spectrum of benzyl penicillin procaine.

causing this absorption at 6.6μ , and the carbonyl oxygen in the ester group of the procaine molecule. Similarly, the nonexistence of this peak in the iron salt suggests the procaine part of the molecule has not been disturbed by the iron coordination.

CONCLUSIONS

The ability to coordinate the spectra of simple compounds of similar structure to more complex

which is present in the spectra of benzyl penicillin procaine and its iron salt suggests that this complex has broken down, although the doublet at 4.0μ is still present, suggesting some type of salt structure is present. Spectra of salts using ferrous iron and ferric salts of benzyl penicillin potassium are ill-defined and suggest considerable impurity present. This work will be continued and results will be reported in a later issue.

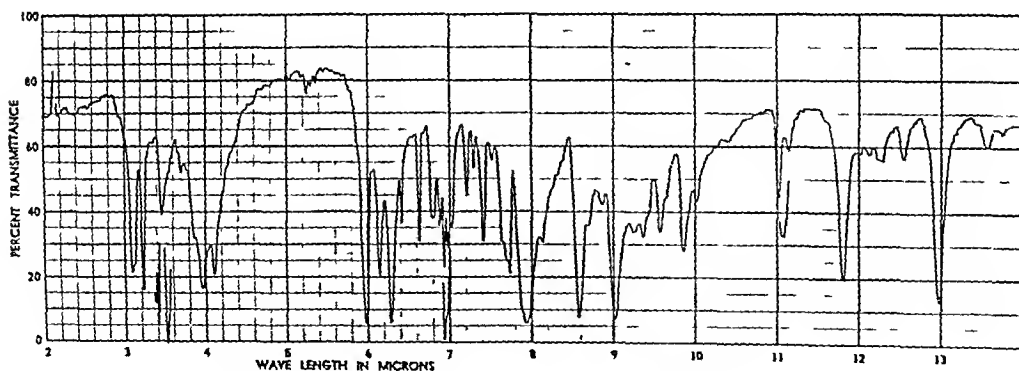


Fig 4 —Infrared spectrum of benzyl penicillin procaine Co.

ones, such as the compound reported here, has made it possible to assign a structure to the compound.

It is intended to further use this technique on other complex salts. An attempt was made to obtain the cobalt salt of benzyl penicillin procaine. The resulting material showed no antibiotic activity and its spectra suggested some breakdown had occurred. The spectra is shown in Fig. 4. The absence of a peak at 5.75μ

REFERENCES

- (1) The Pharmacopeia of the United States of America, 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 942
- (2) U. S. pat. 2,669,563
- (3) Federal Register and the Code of Federal Regulations, Title 21, Chap. 1, Parts 141 a, b, c, d, e (1954)
- (4) Jadassohn, W., Boymond, P., and Isler, H., *Rev. méd. Suisse romande*, 67, 788 (1947)
- (5) Brande, E. A., and Nachod, F. C., "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, 1955, p. 218

The Pharmacology of Some New Cyclooctylalkylamines I.*

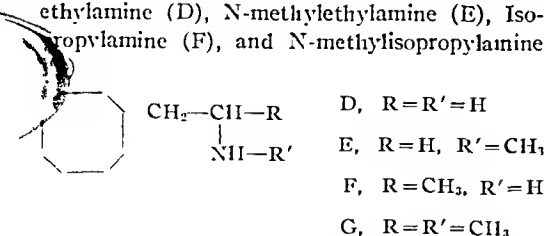
Vasopressor Activity and the Effect on the Heart Rate

By RAYMOND J. KAHL, WILLIAM E. JOHNSON, and DAVID W. O'DAY

Cardiovascular responses to four new cyclooctylalkylamines have been determined using dogs as the experimental animals. The compounds tested were β -cyclooctylethylamine, N-methyl- β -cyclooctylethylamine, β -cyclooctylisopropylamine, and N-methyl- β -cyclooctylisopropylamine. Epinephrine hydrochloride and two structural homologs, Clopane® and Benzedrex®, were used as standards for comparison. The new compounds were found to be somewhat less active as vasopressor agents than Clopane and Benzedrex when compared with epinephrine hydrochloride. All four cyclooctylalkylamines showed an increase in heart rate. This increase ranged from moderate to rather marked and was sustained for at least four minutes. The vasodepressor activity of the new compounds was found to be about the same as that of Clopane and Benzedrex when these compounds were injected following the administration of the adrenergic blocking agent Dibenamine.®

SINCE the appearance on the market of two cycloalkylalkylamines, namely, cyclopentamine, Clopane containing a five-membered ring, and propylhexedrine, Benzedrex, containing a six-membered ring, an attempt has been made to further correlate cycloaliphatic ring size with sympathomimetic activity. McCarthy and Brown (1) prepared four compounds containing the seven-membered ring with the ethylamine and isopropylamine side chain and their respective N-methyl homologs. The pharmacological evaluation of these compounds is not yet available.

We are reporting herewith the first of a series of papers on the pharmacological evaluation of four new amines (2) containing the eight-membered ring with two and three-carbon side chains: ethylamine (D), N-methylethylamine (E), Isopropylamine (F), and N-methylisopropylamine



The work herein described dealt with the vasopressor activity of these cyclooctylalkylamines before and after the administration of the adrenergic blocking agent Dibenamine. The change in heart rate affected by the intravenous administration of these compounds was also recorded.

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The authors are indebted to Smith, Kline and French Laboratories of Philadelphia, Pa., for the Benzedrex® and to Eli Lilly Company of Indianapolis, Ind., for the Clopane® used in this study.

EXPERIMENTAL

Dogs that ranged from 6.6 to 16.4 Kg. in body weight and of random sex were used for blood pressure, respiration, and cardiac rate recordings. The animals were anesthetized with 45 mg./Kg. of intraperitoneal pentobarbital sodium. A total of 8 male and 15 female dogs were used in the determination of pressor responses to the new compounds and to the selected standards. Heart rate recordings were obtained on a total of 7 dogs.

Each anesthetized animal was secured to the operating table in a supine position. The trachea and left common carotid artery were exposed through a midline cervical incision. A cannula was inserted into the trachea and connected directly to an air tambour to record respiratory movements. A glass arterial cannula was secured in the exposed common carotid artery, and connected directly to a mercury manometer by means of a polyethylene tube that was filled with a 10% sodium citrate solution. The solution acted as an anticoagulant to prevent the formation of blood clots in the cannula. All the intravenous administrations were made through a cannula which was secured in the femoral vein. The compounds were washed into the circulation with 5 ml. of normal saline solution from a buret that was connected to this cannula. The heart rate was determined by means of a stethoscope and was recorded on a rapidly turning kymograph drum.

Epinephrine HCl in doses of 0.01 mg./Kg. was selected as a primary standard for comparison. It was administered at the beginning and at the end of each experiment to ascertain the reactivity of the animal. Clopane and Benzedrex were used as additional standards for comparison. They were administered in doses of 1.0 mg./Kg. calculated on the basis of the hydrochloride salt. The new amines were given in doses of 1.0 mg./Kg. calculated on the basis of the free amine but were administered as the hydrochloride salt.

Dibenamine in doses of 15 mg./Kg. was used as a sympathetic blocking agent to determine if the vasopressor activity of the new compounds could be reversed by such an agent. After the administration

tion of Dibenamine, forty-five to sixty minutes were allowed to elapse before the injection of this amine. This procedure permitted sufficient time for a sympathetic blockade to be established.

In all cases the blood pressure was allowed to return to a constant level before another test compound was administered. This level of blood pressure corresponded favorably to the normal blood pressure of the animal.

RESULTS AND DISCUSSION

Table I contains the pressor responses, showing the elevations in pressure, as expressed in mm. of Hg, that were produced in dogs by the intravenous administration of the new compounds and the standards. It should be noted that all the compounds tested were less than 0.0072 times as active as pressor agents as was epinephrine HCl under the conditions of the experiments. The pressor activity of these compounds shows that they decrease in potency in the following order: epinephrine HCl, Clopane HCl, Benzedrex HCl, N-methyl- β -cyclooctylisopropylamine, β -cyclooctylethylamine, N-methyl- β -cyclooctylethylamine, and β -cyclooctylisopropylamine. Figure 1 shows kymograph recordings of typical pressor responses that were produced on

dogs by the intravenous administration of these compounds.

Clopane usually produced the most prolonged pressor responses. The duration of activity of the remaining compounds decreased in the following order: N-methyl- β -cyclooctylisopropylamine, Benzedrex, β -cyclooctylisopropylamine, β -cyclooctylethylamine, N-methyl- β -cyclooctylethylamine, and epinephrine HCl. Table II contains the individual and average readings for the duration of pressor action on each animal. The table also presents averages for each drug when tested on a number of animals.

Intravenous administration of Dibenamine HCl reversed the pressor action of all the new compounds and the standards. This particular phase of the evaluation was performed on a limited number of animals, and only a qualitative determination can be reported. Figure 2 contains kymo-

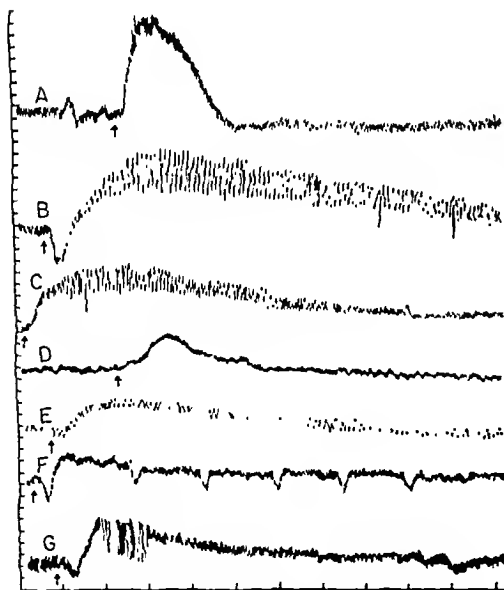


Fig. 1.—Typical pressor responses produced on dogs by the intravenous injection of the hydrochloride salt of the following compounds: A = Epinephrine HCl, 0.01 mg./Kg.; B = Clopane HCl, 1.0 mg./Kg.; C = Benzedrex HCl, 1.0 mg./Kg.; D = β -cyclooctylethylamine HCl, 1.0 mg./Kg.; E = N-methyl- β -cyclooctylethylamine HCl, 1.0 mg./Kg.; F = β -cyclooctylisopropylamine HCl, 1.0 mg./Kg.; and G = N-methyl- β -cyclooctylisopropylamine. Each division on the vertical scale equals 10 mm. of Hg, and each division on the horizontal scale is equivalent to one minute of elapsed time.

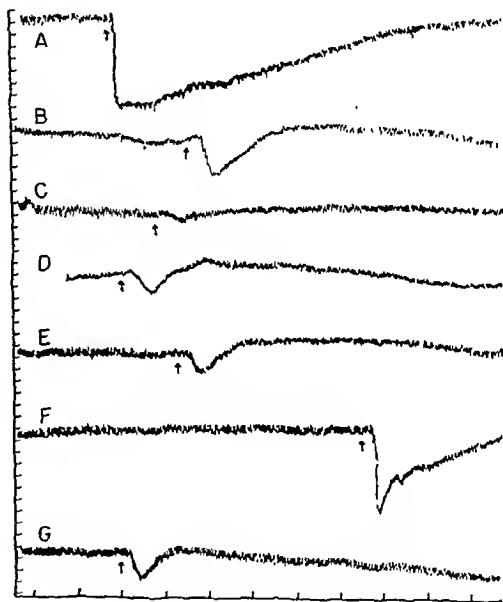


Fig. 2.—Typical depressor responses produced on dogs by the intravenous injection of the hydrochloride salt of the following compounds after the administration of the adrenergic blocking agent Dibenamine. A = Epinephrine HCl, 0.01 mg./Kg., B = Clopane HCl, 1.0 mg./Kg., C = Benzedrex HCl, 1.0 mg./Kg., D = β -cyclooctylethylamine HCl, 1.0 mg./Kg., E = N-methyl- β -cyclooctylethylamine HCl, 1.0 mg./Kg., F = β -cyclooctylisopropylamine HCl, 1.0 mg./Kg., and G = N-methyl- β -cyclooctylisopropylamine. Each division on the vertical scale equals 10 mm. of Hg, and each division of the horizontal scale is equivalent to one minute of elapsed time.

graph recordings of depressor responses that were obtained by the administration of the test compounds after the administration of Dibenamine HCl.

Respiratory responses to the 1.0 mg./Kg. doses of the new compounds were not uniform. Only

TABLE 1—COMPARATIVE PRETOR RESPONSES OF THE NEW CYCLOOCTYLALKYLAMINES AND THE SELECTED STANDARDS

Dog No.	Sex	Propylamine Hydrochloride			Clopine Hydrochloride			Benzedrex Hydrochloride			and D ₁ ^b			Compound D ^c			Compound F ^d			Compound G ^e					
		A	B	Av	A	B	C	Av	A	B	C	Av	A	B	C	Av	A	B	C	A	B	C	Av		
1	M	58	60	59									15		15		20	17	14	17					
2	F	29	30	25									30	31	25	29	30	30							
3	F	28	38	33									23		23		38	26	26	30					
4	M	15	51	50									27		27		23				33	20	27		
5	F	17	58	53																	38		38		
6	M	19	73	81																	15	11	8	11	
7	F	15	62	76					88	16	52										20		20		
8	F	76	58						50	30	40										18	14	16	16	
9	F	109							78		78										32		32		
10	M	65							64		64													36	
11	F	76																							
12	F	81																							
13	M	18	102	75																					
14	F	106	110						64	30	15	36													
15	F	68	90	106					66	19	43														
16	M	92	83	87					66	36	23	42													
17	F	138	136	128																					
18	F	134		134					84		84														
19	F	82	100	91																					
20	F	116	100	123					46		46														
21	M	68	71	71					11		11														
22	F	77	90	83					22		22														
23	M	70	88	79																					
Average all Animals				74.8					53	66		41	7				22	6						38	8

^a The letters A, B, and C denote successive doses of the compound administered to any given dog and the average response is recorded in the column labeled Av. Doses of the new compounds were calculated on the basis of the free amines and were administered as the hydrochloride salts.

^b Compound D = N-methyl-β-cyclooctylethylamine. ^c Compound E = β-cyclooctylisopropylamine.

^d Compound F = N-methyl-β-cyclooctylethylamine. ^e Compound G = N-methyl-β-cyclooctylethylamine. The pretor responses are in terms of mm. of Hg, over the normal blood pressure of the animal.

TABLE II.—DURATION OF PRESSOR ACTION OF THE NEW CYCLOOCTALKYLAMINES AND THE SELECTED STANDARDS

Dog No	Sex	Epinephrine Hydrochloride —0.01 mg/Kg—				Clonidine Hydrochloride —1 mg/Kg—				Benzhex Hydrochloride —1 mg/Kg—				Compound D c —1 mg/Kg—				Compound E d —1 mg/Kg—				Compound F e —1 mg/Kg—				Compound G f —1 mg/Kg—			
		A ^b	B	C	Av	A	B	C	Av	A	B	C	Av	A	B	C	Av	A	B	C	Av	A	B	C	Av				
1	M	1 4 ^a	3 2	1 9	2 17																								
2	F	1 3	1 1	1 8	1 40																								
3	F	1 3	1 4	1 1	1 27																								
4	M	3 1	2 7	3 0	2 93									3 0	5 0	3 0	3 67												
5	F	3 5	5 0	5 0	4 50									5 0		5 00													
														4 5		4 50													
6	M	1 0	4 5	5 0	3 50																								
7	F	1 8	2 4	2 5	2 23					14	5 6	5	10 50																
8	F	2 2	3 3		2 85					9	5 10		9 75																
9	F	4 0			4 00					8	5		8 50																
10	M	1 3			1 30					8	0		8 00																
11	F	1 2			1 20																								
12	F	2 2			2 20																								
13	M	0 9	1 5		1 20									7 7	4 0	4 0	5 23												
14	F	1 2	1 2		1 20									4 3	3 0		3 65												
15	F	1 2	1 5	2 0	1 57									7 0	6 8	5 8	6 53												
16	M	1 4	2 3		1 85																								
17	F	2 2	3 0	3 0	2 73					2 7	1 2	0 7	1 53																
18	F	1 6			1 60					1	3		1 30																
19	F	3 0	3 6		3 30									8 0			8 00												
20	F	1 5			1 50									3 6			3 60												
21	M	1 6	1 7		1 65									2 0			2 00												
22	F	1 5	1 7		1 60																								
23	M	1 3			1 30									5 2	3 2		4 20												
Average all animals					2 13					8 55				5 15			4 21			3 72		4 97			6 23				

^a The figures represent the minutes required for the blood pressure to return to normal after each injection

^b The letters A, B, and C denote the successive doses of the compound administered to any given dog, and the average response is recorded in the column labeled Av

^c Compound D = β -cyclooctylethylamine ^d Compound E = N-methyl- β -cyclooctylethylamine ^e Compound F = β -cyclooctylisopropylamine ^f Compound G = N-methyl- β -cyclooctylisopropylamine

minor variations in the respiratory rate and amplitude could be detected.

The figures in Table I reveal a striking difference in the tachyphylaxis produced by the cyclooctylalkylamines when compared with Clopane and Benzedrex. The inability of subsequent doses to produce a response approximating the initial pressor reaction was much more pronounced in the case of Clopane and Benzedrex.

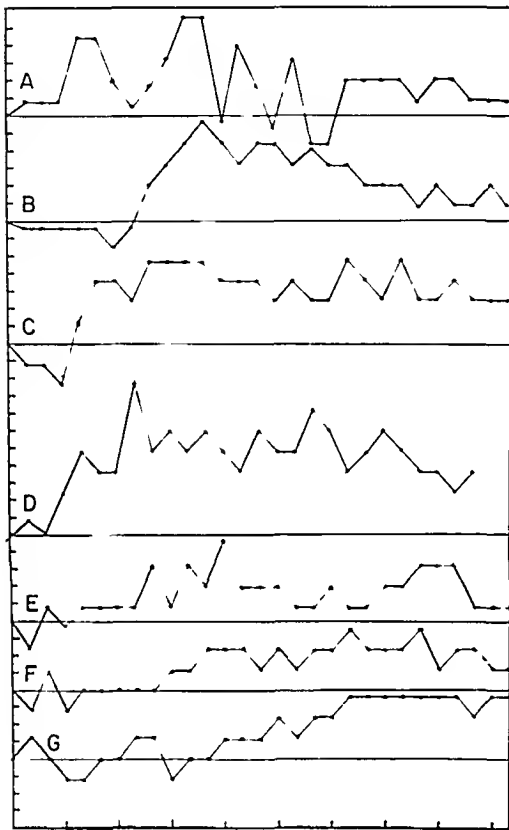


Fig. 3.—Changes in heart rate produced on dogs by the intravenous injection of the standards and the cyclooctylalkylamines. The dose in each case was 1.0 mg./Kg., except epinephrine HCl which was 0.01 mg./Kg. A = Epinephrine HCl, B = Clopane HCl, C = Benzedrex, D = β -cyclooctylethylamine, E = N-methyl- β -cyclooctylethylamine, F = β -cyclooctylisopropylamine, and G = N-methyl- β -cyclooctylisopropylamine. The horizontal lines across the figure represent the normal heart rate in each case. Displacement of the curves above and below these lines represent an increase or decrease in heart rate respectively. Each division on the vertical scale represents a change in heart rate of 5 beats per minute. Each division on the horizontal scale at the bottom of the figure equals an elapsed time of thirty seconds.

Heart rate changes that resulted from the administration of the evaluated compounds are graphically presented in Fig. 3. β -Cyclooctylethylamine was found to be the most potent cardiac accelerator of the new compounds. Clopane and Benzedrex were slightly less potent. The remaining three cyclooctylalkylamines were considerably less active than any of the other compounds that were tested.

SUMMARY

1. Certain cardiovascular responses to four recently synthesized sympathomimetic amines (N-methyl- β -cyclooctylethylamine, β -cyclooctylethylamine, N-methyl- β -cyclooctylisopropylamine, and β -cyclooctylisopropylamine) have been evaluated on dogs. Epinephrine HCl, Clopane HCl, and Benzedrex HCl were used as standards for comparison.

2. The compounds can be listed in order of decreasing pressor potency as epinephrine HCl, Clopane HCl, Benzedrex HCl, N-methyl- β -cyclooctylisopropylamine, β -cyclooctylethylamine, N-methyl- β -cyclooctylethylamine, and β -cyclooctylisopropylamine.

3. When listed in order of decreasing duration of action, the compounds are arranged as Clopane HCl, N-methyl- β -cyclooctylisopropylamine, Benzedrex HCl, β -cyclooctylisopropylamine, β -cyclooctylethylamine, N-methyl- β -cyclooctylethylamine, and epinephrine HCl.

4. The sympathetic blocking agent, Dibenzamine HCl, reversed the pressor action of all the new compounds and the standards.

5. The new compounds produced no significant changes in respiratory rate and amplitude in the 1.0 mg./Kg. doses that were used.

6. The new compounds produced considerably less tachyphylactic effect on blood pressure than did Clopane HCl and Benzedrex HCl.

7. In 1.0 mg./Kg. doses the compounds produced cardiac accelerator activity in the following order of decreasing potency: β -cyclooctylethylamine, Clopane and Benzedrex, N-methyl- β -cyclooctylethylamine, N-methyl- β -cyclooctylisopropylamine, and β -cyclooctylisopropylamine. In 0.01 mg./Kg. doses epinephrine HCl was about equal in cardio-accelerator potency to the 1.0 mg./Kg. doses of Clopane and Benzedrex.

REFERENCES

- (1) McCarthy, W. C., and Brown, T. H., *This Journal*, **43**, 661 (1954).
- (2) McCarthy, W. C., and Kahl, R. J., *J. Org. Chem.*, **21**, 985 (1956).

Tragacanth Solutions I.

The Relation of Method of Preparation to the Viscosity and Stability*

By GERHARD LEVY† and T. W. SCHWARZ

Various factors which influence the viscosity of tragacanth solutions are discussed. The effects of homogenization and heat on solutions of the gum are reported. The degree of initial hydration of the gum is related to the viscosity changes that occur during aging. This viscosity may first increase and then decrease, or it may solely decrease. Studies of the viscosity changes that occur over relatively long periods of time show that the magnitude of such changes may be predicted by methods which are outlined in this paper.

TRAGACANTH is the dried gummy exudation from Asiatic species of *Astragalus* (Fam. Leguminosae). It is a hydrophilic colloid of carbohydrate nature (1, 2). One of the oldest gums known to man, it was described by Theophrastus several centuries before Christ; and it has been official in every edition of the United States Pharmacopeia since 1820.

Tragacanth has a long history and even today it is one of the most important gums used in pharmacy. It is employed as a thickening, suspending, and stabilizing agent in jellies, water-soluble ointments, emulsions, suspensions, and mucilages. It is used in ephedrine sulfate jelly N. F. X, in methylosaniline chloride jelly, N. F. IX, in thimerosal jelly N. F. IX, and in a large number of nonofficial products. The fact that six out of the seven contraceptive jellies listed in New and Nonofficial Remedies 1955¹ include tragacanth as a thickening agent, is an indication of its wide acceptance in industry. The present-day importance of the gum is evidenced by two recent reports from the laboratories of major pharmaceutical houses (3, 4) which use it as the thickening and suspending agent in new drug formulations.

Despite the time-tested qualities of tragacanth, it shares some of the disadvantages inherent in the use of all natural gums. It is a relatively crude natural product, whose chemical constitution and thickening capacity are influenced by climatic changes and methods of harvesting. Consequently, products that contain tragacanth as the thickening agent may vary in consistency and in aging characteristics (5-10).

Numerous investigators have reported that the

viscosity of tragacanth solutions is influenced by the method of preparation, pH, and storage temperature. The gum appears to be composed of two different constituents, one soluble in water, the other swelling but not dissolving in water (11). Many investigators found that the viscosity of tragacanth solutions increases markedly with time (1, 7-10, 12). Chambers (13) attributed this viscosity increase to a slow process of hydration. He regarded a recently prepared solution as being only partially hydrated, consisting of incompletely hydrated globules, which may be likened to the discontinuous phase of a two-phase system dispersed in a continuous phase of dissolved and hydrated material. In time, some of the swollen particles of the discontinuous phase go into solution, while the already dissolved gum slowly undergoes degradation (14).

The two processes, hydration and degradation, which occur simultaneously during aging, result in viscosity changes that have been difficult to predict and which have made the commercial preparation of tragacanth solutions with uniform properties a somewhat specialized and empirical endeavor.

Since most freshly prepared tragacanth solutions are only partially hydrated, their viscosity will rise as hydration proceeds. When the "depot" of nonhydrated gum is exhausted, no further viscosity increase will occur. Simultaneously, the hydrated gum undergoes degradation. As more gum goes in solution, it too undergoes degradation. Thus the viscosity decrease due to breakdown gradually exceeds the viscosity increase due to continuing hydration. When the rate of hydration equals that of degradation, the viscosity of the solution remains constant; and during this phase of the aging process the viscosity will be at its maximum. After tragacanth is fully hydrated, the viscosity begins to decrease markedly because the con-

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¹ Subsequent editions of N. N. R. did not list the composition of the jellies.

tinuing degradation of the gum is no longer offset by further hydration

This study was undertaken to attempt the evaluation of the factors that contribute to the hydration as well as to the degradation of tragacanth in aqueous solution. Solutions in which the gum is completely hydrated yet little decomposed should exhibit the highest viscosity. Thus, if the degree of hydration could be regulated without causing simultaneous degradation, it should be possible to prepare solutions in which the following properties may be controlled and adjusted to the specific requirements of the product: (a) initial viscosity, (b) maximum viscosity which the preparation will attain, (c) the time at which the viscosity is fairly constant, whether initially, or after varying periods of storage, (d) the length of time during which a minimum effective viscosity is maintained.

These properties are of great importance in pharmaceutical formulation. For this reason, the investigation aimed at developing a method for obtaining maximum hydration of tragacanth with little or no simultaneous degradation. Two main approaches were followed: homogenization (mechanical treatment) and application of heat.

In addition, accelerated aging tests were carried out on tragacanth solutions prepared by different methods in order to obtain further knowledge about the effect of such procedures on the long term viscosity changes of the gum.

EXPERIMENTAL

Preparation of Samples.—All samples were prepared with tragacanth gum, U. S. P. ribbon No. 1. The solutions were prepared by adding the gum to 50 ml. portions of preserved water (methylparaben, S. P., 0.1%, propylparaben, U. S. P., 0.025%, distilled water *q. s.*) contained in an eight ounce wide mouth glass jar. The samples were then agitated by a mechanical shaker for twenty-four hours.

Viscosity Measurement.—Samples of the tragacanth solutions were placed in 50 ml. centrifuge tubes and occluded air was removed by centrifugation at 2,000 r. p. m. for ten minutes. The samples were then brought to a temperature of $25^{\circ} \pm 0.5^{\circ}$ in a constant temperature bath. The viscosity was determined with the Brookfield Synchro Lectric Viscometer, Model LVT, using spindle No. 4 at 6 r. p. m. Readings were taken after ten minutes of spindle rotation.

Homogenization.—Homogenizations were carried out with a single piston hand homogenizer² or with a Charlotte Colloid Mill, Model A. The clearing in each piece of equipment was kept constant; the nozzle on the hand homogenizer was kept one turn short of being completely tightened and the clear-

ance on the colloid mill was adjusted to 0.0025 inches.

Stability Test.—Stability tests were made by storing the samples in eight ounce glass jars at a temperature of $50^{\circ} \pm 1^{\circ}$. Samples were removed periodically and their viscosity was determined after storing for twenty-four hours in a water bath at 25° .

All experiments were carried out in duplicate and the data in the graphs are average values.

RESULTS AND DISCUSSION

The Effect of Homogenization.—A 1.5% tragacanth solution prepared two days earlier was passed repeatedly through a hand homogenizer. Samples were collected after each passage and the viscosity of these samples was determined after twenty-four hours, as previously described.

Another solution of 1.5% tragacanth was passed repeatedly through a colloid mill. Samples were collected after each passage and their viscosity was determined twenty-four hours later. The results are shown in Fig. 1.

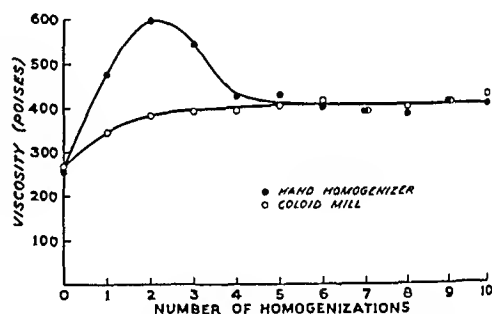


Fig. 1—The effect of homogenization on the viscosity of a 1.5% tragacanth solution.

The graph indicates the viscosity increase of the tragacanth solutions as a result of homogenization. Both solutions eventually reached the same viscosity plateau, where additional passages through the hand homogenizer or the colloid mill caused no further viscosity change. It is significant that the solution which was passed through the hand homogenizer reached a viscosity peak before attaining the constant lower viscosity level.

The effect of homogenization in increasing the viscosity of tragacanth solutions has been previously reported (13, 15) but as far as the authors could establish, this is the first instance in which the attainment of a viscosity plateau after repeated homogenizations is described.

It is important to note that the solution which underwent treatment with a hand homogenizer reached the same viscosity plateau as the solution which was subjected to shear by the colloid mill.

The homogenization of tragacanth solutions causes a reduction in the size of the dispersed globules of partially hydrated gum. The increased surface area exposed to the water brings about a

* Distributed by the Central Scientific Company, Chicago 13, Ill.

greater hydration of the gum and as a result the viscosity of the solution is increased. It has been found previously and follows from basic concepts of colloid chemistry that, for example, milk and clay suspensions increase in viscosity as a result of homogenization. Philippoff (16) attributes this effect to a better solvation of the particles as a consequence of particle size reduction. It is likely that the same considerations apply to the effects of the homogenization of tragacanth solutions.

The presence of the hump in the viscosity curve of the solution which was passed through the hand homogenizer introduces an interesting problem of interpretation on the nature of the internal structure of tragacanth solutions. Philippoff (16) states that mechanical treatment of gel-forming substances may cause a decrease in their viscosity. Such a decrease may be partly reversible and partly irreversible. He distinguishes between "labile" structural viscosity and "stable" structural viscosity. The former is sensitive to mechanical treatment while the latter is not.

The hump, which is evident in the viscosity curve of the solution which was passed through the hand homogenizer, may be an indication of "labile" structural viscosity. Several passages through the hand homogenizer were required to completely destroy this "labile" component. On the other hand, the absence of the viscosity hump in the solution which was passed through the colloid mill may be attributed to the much more severe mechanical treatment which that solution underwent. Evidently, the labile component was completely destroyed by the first passage through the colloid mill.

Considering the chemical nature of tragacanth, a compound of high molecular weight containing many polar groups, it is possible that mechanical treatment may cause a rupture of hydrogen-bonds which bind the gum molecules to each other. Once such bonds are broken, water molecules may replace gum molecules so that individual gum molecules are bound to water alone instead of to each other. After the "labile" viscosity component was thus destroyed, only the "stable" viscosity component remained, which apparently is not affected by the mechanical treatment and which accounts for the viscosity plateau.

The Effect of Heat.—The effect of subjecting tragacanth solutions to graded amounts of heat in the course of their preparation is shown in Fig. 2. In this experiment, beakers containing 200 ml. of preserved water were placed in a boiling water bath. When the preserved water began to boil, the tragacanth was added and the solution was agitated with an electric stirrer. After boiling the solutions for the indicated periods, they were removed from the water bath and permitted to come to room temperature. The water lost by evaporation was then replaced, the solutions were agitated in a mechanical shaker for two hours, and placed in a water bath at 25°. On the next day, the viscosity of the solutions was determined in the usual manner.

The graph shows that boiling for periods longer than fifteen minutes caused a considerable decrease in the viscosity of the solutions, which indicates more pronounced degradation than hydration. Since at least thirty minutes of boiling were required to dissolve the tragacanth ribbons, it is obvious that the application of sufficient heat to expedite solu-

tion results in considerable degradation of the gum.

Tragacanth solutions subjected to graded amounts of heat were repeatedly passed through the hand homogenizer as previously described. Their viscosity was determined after each homogenization. The results are presented in Fig. 3. The solutions that had been heated for the longest period of time had the lowest viscosity plateau. Their initial viscosity hump also became less evident and disappeared entirely in the sample that was heated for sixty minutes.

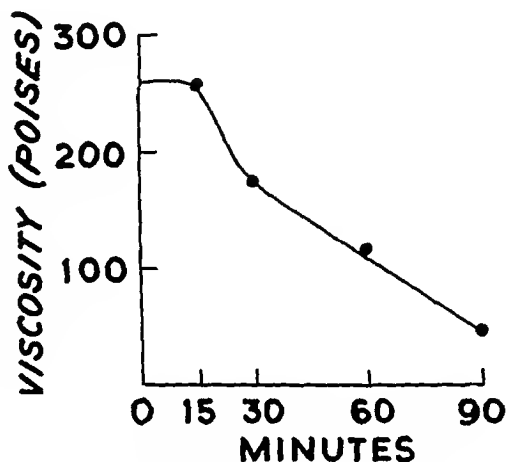


Fig. 2.—The effect of heat (100°) used in the preparation of 1.5% tragacanth solutions on their viscosity.

These observations illustrate the effect of heat on both the "labile" and "stable" structural viscosity of tragacanth. Heat has probably three effects: it causes degradation of the gum, it ruptures hydrogen bonds, and it increases the rate of hydration of tragacanth. Obviously, the lowering of the viscosity plateaus in the heat-treated solutions reflects the known chemical degradation of the gum by heat.

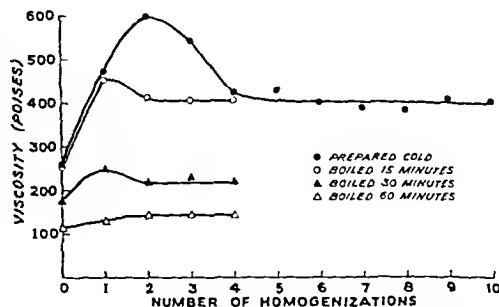


Fig. 3.—The effects of heat and homogenization on the viscosity of 1.5% tragacanth solutions.

Gralen and Karrholm (14) were able to show this degradation by determining the average molecular weight of tragacanth in solution before and after boiling. They report an average molecular weight change from 840,000 to 550,000 in the samples which they had boiled for thirty minutes. After boiling a tragacanth solution for three hundred and thirty minutes, the average molecular weight was reduced to 350,000.

The rupture of hydrogen bonds between gum molecules by heat is probably responsible for the decreasing prominence of the viscosity hump.

Some reports appearing in the literature state that short periods of boiling, usually two to fifteen minutes, increase the viscosity of tragacanth solutions (14-16). These are examples of the use of heat to increase the rate of hydration. It is quite probable that small increments of heat may promote the hydration of tragacanth while having only a negligible degradative effect. However, a number of variables must be taken into consideration here, such as the amount of heat applied, the ratio of hydrated to unhydrated gum at the time heating was instituted, and the volume of the solution. Such variables account for the almost unpredictable effect of heat on tragacanth solutions.

At least one investigator (17) concluded that the application of heat to tragacanth mucilage to increase viscosity must be rejected.

The Effect of Aging.—The mechanism of the viscosity changes which tragacanth solutions undergo during aging was discussed in the beginning of this paper. Figure 4 shows the viscosity curve for homogenized, nonhomogenized, and "half-homogen-

ized" gum. The graph indicates that the viscosity of the homogenized solution, after a very small initial increase, showed very little change for about two weeks. After twenty-one days, the viscosity began to decrease rapidly. Since the initial viscosity of the solution showed very little further increase, it can be assumed that the gum was almost completely hydrated by homogenization.

The viscosity of the nonhomogenized gum increased with time as expected. It reached a maximum in thirty-one days at 50°. From then on the viscosity began to decrease.

The "half-homogenized" solution illustrates how controlled partial homogenization may be used to obtain intermediate viscosity effects. The magnitude of the viscosity changes that occurred in the test period was much smaller with the "half-homogenized" gum than with the other two solutions.

These observations suggest that methods for preparing aqueous tragacanth solutions may be specifically designed to obtain products of different stability characteristics.

Solutions intended for relatively short storage periods may be completely homogenized. Such solutions will show no significant viscosity gain and little viscosity decrease for some time.

Solutions intended for long periods of storage may be prepared by dissolving the tragacanth in cold water without subsequent homogenization. Such solutions increase in viscosity on aging. A viscosity drop occurs only after relatively long periods of time. The approximate maximum viscosity which such solutions can reach, may be predicted by homogenization of a sample.

Solutions intended for intermediate periods of storage may be partially homogenized. The viscosity changes of such solutions may be regulated by varying the ratio of homogenized to nonhomogenized gum in accordance with previously stated considerations.

REFERENCES

- (1) Mantell, C. L., "Water-Soluble Gums," Reinhold Publishing Corporation, New York, 1947, pp. 63-69.
- (2) Beach, D. C., "Natural Plant Hydrocolloids," Am. Chem. Soc., Washington, D. C., 1954, pp. 38-13.
- (3) Sabatini, G. R. and Gulesich, J. J., *THIS JOURNAL*, *Pract. Ed.*, 17, 806(1956).
- (4) Garrett, E. R., *THIS JOURNAL*, 45, 171(1956).
- (5) Kaufman, J. W., Burlage, H. M., and Lloyd, W. R., *ibid.*, *Pract. Ed.*, 16, 234(1955).
- (6) Martin, A. N., "American Pharmacy," 11th ed., J. B. Lippincott Company, Philadelphia, 1955, p. 271.
- (7) Brindle, H. and Burlinson, H., *Quart. J. Pharm. and Pharmacol.*, 7, 492(1934).
- (8) Osborne, G. E., and Lee, C. O., *Quart. Nat'l. Formulary Committee*, 19, 12(1951).
- (9) Nichols, A. B., *THIS JOURNAL*, 26, 823(1937).
- (10) Nichols, A. B., *THIS JOURNAL*, 28, 98(1939).
- (11) "The Dispensatory of the United States," 25th ed., J. B. Lippincott Company, Philadelphia, 1955, pp. 1112-1445.
- (12) Gabel, L. F., *THIS JOURNAL*, 23, 311(1931).
- (13) Chambers, W. P., *Quart. J. Pharm. and Pharmacol.*, 21, 44(1948).
- (14) Gralen, N., and Karrholm, M., *J. Colloid Sci.*, 5, 21(1950).
- (15) Middleton, G., *Quart. J. Pharm. and Pharmacol.*, 9, 493(1936).
- (16) Philippoff, W., "Viskosität der Kolloide," Verlag Steinkopff, Dresden, Germany, 1942, pp. 317-322.
- (17) Kleinert, R., *Pharm. Ztg. Berl.*, 81, 323(1936).

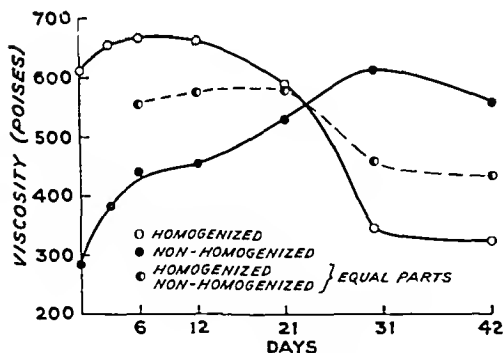


Fig. 4.—The effect of homogenization on the aging characteristics of 1.5% tragacanth solutions (storage temperature 50°).

ized" solutions of tragacanth which were kept at 50°. The homogenized solution was passed twice through a hand homogenizer to reach the viscosity peak. The "half-homogenized" solution was made by mixing in a large jar equal parts of homogenized

The Role of Residual Calcium in the Viscosity Changes of Sodium Alginate Solutions*

GERHARD LEVY† and T. W. SCHWARZ

The marked viscosity increase of most sodium alginate solutions after freezing and thawing is due to and proportional to the amount of residual calcium present. The rheological character of such solutions is also altered as a result of freezing. Sequestering agents and polyols affect the initial viscosity and modify the effects of freezing.

THE AUTHORS have recently reported (1) that solutions of a type of sodium alginate exhibit a marked viscosity increase after freezing and thawing. This behavior, which is apparent in most commercial types of sodium alginate, was found to be absent in highly purified material, which had a very low calcium content (2). This report presents the results of an investigation of the role of residual calcium in the viscosity changes of sodium alginate solutions, especially after freezing and thawing.

The major manufacturing processes for sodium alginate involve the precipitation of calcium alginate by addition of a soluble calcium salt such as calcium chloride to the crude algin. The calcium alginate is subsequently converted to alginic acid and then to sodium alginate (3, 4). The sodium alginate of commerce contains varying amounts of residual calcium, depending, among other factors, on the number of washings of the alginate with hydrochloric acid solution and the origin of the kelp. Since the severity of the extraction process required to obtain highly purified sodium alginate reduces the thickening capacity and increases cost, most commercial products contain appreciable amounts of residual calcium.

EXPERIMENTAL

Materials.—Five types of pharmaceutical grade sodium alginate obtained from three different manufacturers were used in this study. Since most manufacturers produce several different types of sodium alginate, no further reference to the source is made.

Methylparaben and propylparaben were U. S. P. grade, while the remaining chemicals were of reagent grade.

Preparation of Solutions.—Solutions of sodium alginate were prepared by sprinkling the powder on 80% of the required amount of preserved water (0.1% methylparaben and 0.025% propylparaben in distilled water) agitated in a Waring Blendor. The solutions were sheared at a definite rate for ten minutes.

Any other component of the solution was dis-

solved in 20% of the required amount of preserved water and added to the sodium alginate solution. The samples were then placed on a mechanical shaker for one hour and stored for three days at room temperature after which the viscosity was determined. When a buffer was used, it was pre-dissolved in the preserved water to which the sodium alginate powder was then added.

Viscosity Determinations.—Viscosity measurements were made with the Brookfield Synchro-Lectric Viscometer, Model LVF. A No. 4 spindle was used for viscosities above 5,000 c. p. s. and a No. 3 spindle for viscosities less than 5,000 c. p. s. The measurements were made at six r. p. m. after ten minutes of spindle rotation on samples which had been adjusted to a temperature of $25^{\circ} \pm 0.5^{\circ}$ in a water bath.

Freezing.—The solutions were stored for twenty-four hours in the freezing compartment of a refrigerator at a temperature of $-16^{\circ} \pm 1^{\circ}$. They were permitted to thaw at room temperature for twenty-four hours and placed in a water bath at 25° for two hours before the viscosity was again determined.

Calcium Determination.¹—Calcium determinations were made with a flame spectrophotometer similar to one described by MacIntyre (5). Calcium absorption was measured at 423 m μ in order to avoid interference by sodium. All samples were previously digested with sulfuric and nitric acids. The calcium content of sodium alginate is reported in terms of the dried powder.

Loss on Drying.—The procedure used was that of the National Formulary, Tenth Edition, for sodium alginate.

Per cent concentration of sodium alginate is reported here, however, in terms of "wet" weight, i. e., before drying.

RESULTS AND DISCUSSION

Table I shows the calcium content of the various types of sodium alginate used in this study as well as the viscosity changes after freezing and thawing. The results indicate that the viscosity increase of sodium alginate solutions after freezing and thawing is proportional to the amount of calcium present.

Figure 1 represents the effect of adding increasing amounts of sodium ethylenediamine tetraacetic acid solution (pH 8.3) to a 1.3% solution of a type of sodium alginate containing 1.3% residual calcium² (Product A in Table I). The solution was

¹ The authors wish to thank Drs. G. S. Gordan and F. Loken of the University of California Medical School for the calcium determinations.

² The two identical figures of 1.3% are coincidental.

* Received September 30, 1957, from the University of California School of Pharmacy, San Francisco 22.

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TABLE I — THE EFFECT OF RESIDUAL CALCIUM ON THE VISCOSITY CHANGES OF SODIUM ALGinate SOLUTIONS AS A RESULT OF FREEZING AND THAWING

Product	Solu- tion %, w/v	Per cent Calcium Manufacturer's Data ^a	Content— Experi- mental ^b	Loss on Drying, ^d %	Viscosity in c p s ^c Before Freezing	After Freezing	Ratio After/ Before
A	2	1.5	1.3	8.7	32,700	87,000	2.7
B	2	1 to 1.5	1.0	7.5	25,900	56,200	2.2
C	3	0.5	0.55	14.6	44,750	78,600	1.8
D	4	0.2	0.15	12.2	42,800	44,200	1.03
E	3	"Low Ca"	0.098	13.7	24,400	24,000	0.98

^a Approximate data. A and D in terms of CaO

^b As Ca⁺⁺ based on dried alginate

^c Average of four samples

^d Average of two determinations

adjusted to a pH of 8.3 with diethylbarbiturate buffer of 0.1 M total concentration to maintain constant sequestering capacity. The arrow on the graph indicates the point at which all the calcium should be sequestered as calculated on the basis of the assayed calcium content.

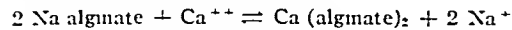
The results show that solutions of sodium alginate below a minimal concentration of unsequestered calcium undergo no viscosity increase due to freezing, and that EDTA can be used to prevent such viscosity changes of sodium alginate solutions containing more than the minimal amount of residual calcium. The addition of sodium citrate or sodium hexametaphosphate instead of EDTA produced the same effect.

Figure 2 illustrates the effect of increasing concentrations of calcium acetate on the viscosities of a 2% solution of a type of sodium alginate containing only 0.098% residual calcium (Product E in Table I). While small amounts of added calcium have practically no effect on the viscosity before freezing, they cause a considerable viscosity increase after freezing and thawing.

It appears that the calcium residue in sodium alginate is present partly as calcium alginate and partly in the form of soluble salts. Gomcz (6) was able to remove part of the calcium by dialysis, indicating the presence of calcium not bound to alginate. On the other hand, the presence of calcium in the form of calcium alginate is indicated by the decrease in viscosity with the addition of increasing amounts of EDTA in sodium alginate solutions containing residual calcium (Fig. 1). This can only occur if the chelating agent substitutes sodium for the calcium which is bound as alginate. The result of this substitution is a decrease in the degree of cross-linking, which is characteristic of calcium alginate.

The viscosity change cannot be ascribed to an electroviscous effect since the concentration of buffer in the solution was sufficient to obliterate this effect. A further support for this assertion is that the addition of EDTA to a solution of highly purified, unbuffered sodium alginate (0.03% Ca) caused no viscosity change whatsoever.

As solutions of sodium alginate undergo freezing and pure ice separates from the solution, the effective concentration of calcium present in soluble form is increased. The equilibrium



is shifted to the right according to the mass law. This increase in calcium alginate concentration causes a greater degree of cross-linking which, in turn, results in a higher viscosity of the solution.

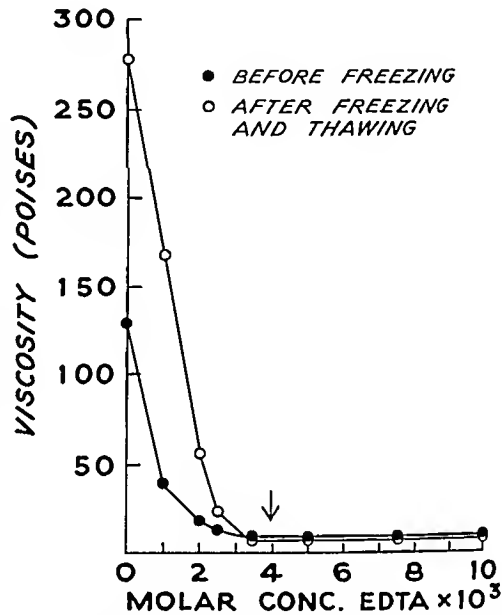


Fig. 1—The effect of EDTA on a 1.3% solution of sodium alginate containing 1.3% residual calcium (Average of three samples)

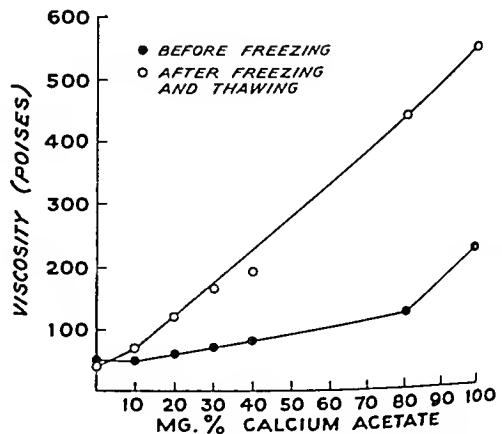


Fig. 2—The effect of added calcium on a 2% solution of sodium alginate of low calcium content (0.098% Ca) (Average of four samples)

After thawing, there is a gradual reversal to the previous equilibrium, but this takes place at a very slow rate (1).

A further indication that the viscosity increase as a result of freezing is due to a concentration of calcium can be seen in the effect which variation of freezing temperature has on the viscosity increase. A solution of sodium alginate which exhibited a viscosity increase of 94% after freezing at -16° had a viscosity increase of only 28% after freezing at -25° , a temperature at which less ice will crystallize out of solution.

In a previous paper (1), it was shown that the presence of glycerin or propylene glycol in sodium alginate solutions containing residual calcium resulted in a smaller or no viscosity increase after freezing and thawing; also, that sodium alginate solutions containing large amounts of added calcium salt showed a negligible viscosity change after freezing and thawing. In both cases there was an increase in the initial viscosity which made any effect of freezing less noticeable. The presence of the hydrophilic polyols results in a partial dehydration of the sodium alginate, probably followed by greater association of the alginate molecules with each other through hydrogen bonds. Furthermore, the presence of polyols lowers the freezing point and; therefore, the enrichment of calcium in the solution due to separation of ice is less than in the absence of polyols, provided the temperature is the same.

In the case of sodium alginate solutions containing large added amounts of calcium, sufficient calcium alginate is formed to give a high degree of cross-linking of alginate molecules. Thus freezing would have little or no further effect on the viscosity.

The formation of calcium alginate in a solution of sodium alginate changes not only the viscosity but also the rheological characteristics of the solution.

While solutions of pure sodium alginate behave like pseudoplastic liquids (7), the presence of calcium alginate in such solutions produces a thixotropic system (8).

The freezing of sodium alginate solutions containing residual calcium causes a twofold change: the ratio of calcium alginate to sodium alginate is increased; and an essentially pseudoplastic system becomes markedly thixotropic. Although the Brookfield Viscometer does not lend itself to a quantitative measurement of thixotropy, it was possible to perceive the presence of a thixotropic component by the decrease in the apparent viscosity as a result of prolonged spindle rotation.

It should be pointed out that the marked viscosity increase of sodium alginate solutions, containing residual calcium, upon freezing and thawing cannot be considered a thixotropic sol-gel transformation. Thixotropic transition must be isothermic, yet the sol-gel change of the solution requires freezing. The change is actually a chemical transformation of part of the sodium alginate to calcium alginate, which imparts the characteristic rheological behavior of the latter to the system. Any interpretation of the viscosity data reported here must take into account this change in rheological properties.

REFERENCES

- (1) Schwarz, T. W., and Levy, G., *THIS JOURNAL*, 46, 562(1957)
- (2) Gibson, K. F., personal communication
- (3) Mantell, C. L., "The Water Soluble Gums," Reinhold Publishing Co., New York, 1947, pp 106-113
- (4) Donnan, F. G., and Rose, R. C., *Can J. Research*, B, 28, 105(1950)
- (5) MacIntyre, I., *Biochem. J.*, 62, part 3, 37(1956)
- (6) Gomez, M. L., *Inst. españ. oceanog., Notas y resúmenes Ser. II No. 74*, 98(1933), quoted by Mantell, *loc. cit.*
- (7) Fischer, E. K., "Colloidal Dispersions," John Wiley and Sons, Inc., New York, 1950, p 172
- (8) Sasaki, T., Miyamoto, S., and Morinaga, Y., *Bull. Chem. Soc. Japan*, 29, 32(1956)

Quantitative Determination of a Series of Malonic Esters by Gas Chromatography*

By J. B. TEPE and H. J. WESSELMAN

Gas chromatography offers a convenient, rapid, and accurate method for analyzing a number of malonic esters which previously were determined less accurately by other physical methods.

FOR MANY YEARS, the production of malonic esters was controlled by means of specific gravity or refractive index measurements. While these methods are reasonably satisfactory for pure compounds or a binary mixture, they fail when applied to mixtures containing three or more components.

With the advent of gas chromatography, it was found highly desirable to apply this technique as a control in the production of malonic esters.¹ Using this method, a mixture of six esters can be determined simultaneously. This technique was first introduced by James and Martin (1) and has been used extensively and applied to a wide range of compounds. Books by Phillips (2) and Keulemans (3) give a very good discussion of the field of gas chromatography.

EXPERIMENTAL

Apparatus.—Gas Chromatograph.—Burrell Kromo-Tog, Model K-2. Column—150 x 0.5-cm glass column with integral heating coil. Column

* Received November 29, 1957, from Eli Lilly and Company, Indianapolis, Ind.

¹ The authors wish to thank Mr. R. W. Bridges for preparing pure samples of the malonic esters used in this work, and Mr. L. D. Seay for assaying the standard mixture of malonic esters.

packed with 20% (w/w) Linde Silicone Oil L-46 (12,500 Centistokes) on Celite C-44857 and operated at 220°. Carrier Gas—Helium at an inlet pressure of 8 lb/sq in. and atmospheric pressure at outlet. Flow rate 50 ml/min. Pipette—Agla Micrometer Syringe, Sample size, 0.01 cc.

Pure samples of the esters to be studied were prepared by laboratory fractional distillation. These samples were chromatographed on various stationary phases. Of these, petrolatum, tri-*m*-cresyl phosphate, and silicone oil proved satisfactory; however, the relatively high temperature (220°) necessary for a good separation caused the petrolatum and tri-*m*-cresyl phosphate to gradually bleed from the column. The silicone column proved to be very stable at this temperature and more than 3,000 samples have been analyzed with this column.

The retention times of the pure esters were determined and a mixture of equal volumes of each ester was then used to calibrate the instrument before the production lots of the various esters were analyzed.

Results are easily reproduced when the operating conditions of the instrument remain constant. In routine analysis, it has proved advantageous to run the standard sample along with each lot of esters being analyzed.

PROCEDURE

The sample to be analyzed is injected into the column through a rubber diaphragm by means of a micrometer syringe. After the sample has passed through the column and the thermal conductivity cell, the chart is removed from the recorder and the amount of each component is calculated. A typical chart for a mixture of six malonic esters is shown in Fig 1.

Since the retention time for each ester is specific under a given set of experimental conditions, each ester can be determined qualitatively very easily. Similarly, the area under each peak is proportional

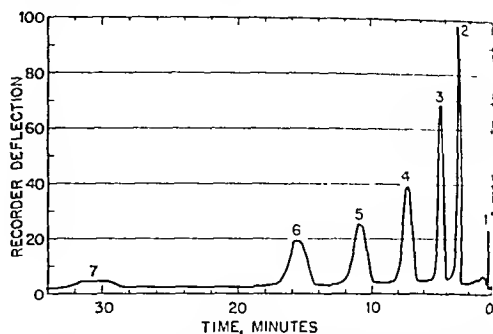


Fig 1—Retention times of six malonic esters using silicone column at 220°. 1—Air, 2—Diethyl malonate, 3—Diethyl ethyl malonate, 4—Diethyl diethyl malonate, 5—Diethyl isoamyl malonate, 6—Diethyl isoamyl ethyl malonate, 7—Diethyl diisoamyl malonate. Sample, 0.01 cc.

(3). When area is used for calculation of relative composition with the assumption that the malonic esters have the same thermal conductivity, the equation becomes:

$$\frac{\text{Area of Individual Peak}}{\text{Sum of Areas of All Peaks}} \times 100 = \text{\% Individual Component in Mixture}$$

Table I shows the results of three separate assays of the standard mixture of malonic esters. Diethyl diisoamyl malonate is not included in this assay as it is not manufactured and does not appear in chromatograms of the production lots of esters.

SUMMARY

A mixture containing several malonic esters can be assayed qualitatively and quantitatively by means of gas chromatography. Without this method, the assay of such a mixture is tedious

TABLE I—TYPICAL ANALYSIS OF A STANDARD MIXTURE OF MALONIC ESTERS

Run No	Area Per cent				
	Diethyl Malonate	Diethyl Ethyl Malonate	Diethyl Diethyl Malonate	Diethyl Isoamyl Malonate	Diethyl Isoamyl Ethyl Malonate
1	11.0	12.2	19.0	22.6	35.0
2	12.8	13.4	18.9	21.5	33.4
3	12.7	12.2	18.9	22.8	33.4
Average	12.2	12.7	18.9	22.3	33.9
Theory weight, %	12.6	12.1	17.8	23.1	34.4
Theory volume, %	11.8	11.8	17.6	23.5	35.3
Theory mole, %	17.1	14.0	17.9	22.0	29.0

to the amount of that constituent in the mixture. Whether this area is proportional to weight per cent or mole per cent is still controversial. In actual practice, this discrepancy can be eliminated by calibrating the system, using either weight per cent or mole per cent.

The areas under the peaks can be measured with a planimeter, by cutting out the peak and weighing, by coupling an integrator to the detector output, or by measuring the peak heights by half-band widths

and time consuming, in addition to being inaccurate at its best.

REFERENCES

- (1) James, A. T. and Martin, A. J. P., *Biochem. J.*, 50, 679 (1952).
- (2) Phillips, C., "Gas Chromatography," Academic Press, Inc., New York, N. Y., 1956.
- (3) Keulemans, A. I. M., "Gas Chromatography," Reinhold Publishing Corp., New York, N. Y., 1957.

Plant Microtechnique: A Note On Rapid Tissue Dehydration*

By ARNOLD C. NEVA

TO THE STUDENT and the research worker, current tissue microtomy in many instances presents sharp contrasts of methods. The standard reference works on microtechnique largely perpetuate the time-consuming conventional methods of dehydration, embedding, and mounting of tissues predominantly for descriptive studies. Conversely, and with noticeably greater frequency during the past decade, the current literature presents a variety of differing techniques, adaptations, improvements, and innovations to both operational functions and intent.

Recurrent emphasis ranges from automatic sectioning and mounting (1, 2) and chelate application (3); to histochemical (4), and rapid one-step dehydration and infiltration operations (5); and to dynamic bioactivity and tissue changes rather than the structural make-up and organization of tissues.

Because modern microtechnique demands an ever increasing number of studies with a minimum of undesirable solvent effects, greater simplification in procedures is the prevailing trend. This also holds true for a better interpretation of structure and organization in tissues as well.

For example, the rapid animal tissue dehydration method with modified Soxhlet extractor, as reported by Saiki and Kling (6), sought to circumvent the time-consuming serial tissue transfer in graded alcohol dilutions and other variants of this long used process.

Without elaborating on initial application trials that will be dealt with in other subsequent detailed reports, the method of Saiki and Kling was not found to be fully applicable to plant tissue work. But with several modifications, the basic principle of rapid tissue dehydration, as outlined by these authors, has been satisfactorily applied to plant tissues.

This time-saving rapid tissue dehydration process can easily be carried out by the following steps, briefly described in the following sequence, and by using whatever modifications may be necessary in individual cases.

The plant material is cut to suitable (approx. 1 square cm.) size and aspirated for approximately one-half hour, or somewhat longer if necessary. Aspiration is carried out in a solution made up of: alcohol, 70 parts; glacial acetic acid, 15 or 10 parts; and water, 20 or 15 parts. Higher glacial acetic acid concentrations may be necessary with tougher woody plant materials; and conversely, it may not be necessary to use the acid at all when working with very soft plant tissues, or ordinary animal tissues.

By successive rinsings, avoiding air inclusion, the aspiration fluid is replaced completely with anhydrous acetone, the dehydrating liquid.

In contrast to the apparatus of Saiki and Kling, a

regular unmodified Soxhlet extraction apparatus is employed with the material for dehydration in acetone placed in a regular extraction thimble loosely covered with cotton. If circumstances necessitate, a suitable variation for a tissue holder can be improvised that prevents the complete solvent run-off from the tissues; if for any reason, this may be objectionable. No difficulty has been encountered thus far in this respect by the writer.

The necessary volume of acetone is poured into the extraction chamber and after assembling, a sufficient excess is added to permit satisfactory continuous operation, and to compensate for any loss of acetone through volatilization.

Approximately 200 Gm. of Drierite¹ is placed into either a 500-cc. or 1-liter receiving flask to serve as a dehydrating agent. The extraction unit is assembled with the receiving flask, and with a cooling condenser. Only enough heat is used to volatilize the acetone, and the dehydration is carried out by the regular Soxhlet extraction procedure.

Satisfactory dehydration of plant tissues is usually accomplished within a two to three-hour period, although certain difficultly dehydrated substances may take somewhat longer.

After dehydration, the anhydrous acetone is replaced with xylol or any of the other regularly used anhydrous solvents; and the microtechnique process is continued with the conventional paraffin embedding, or with other similar methods as desired.

Fresh plant tissues have been satisfactorily processed into paraffin for sectioning in one day by the method described.

This significant saving of time is not the only advantage of rapid tissue dehydration achieved, although it is the principal one. For example, interesting histochemical fixations have resulted in studies using the acetone rapid dehydration method. Certain cell content precipitations and localizations, the nature of which is yet undetermined, have been witnessed in a particular leaf tissue. Also, just as other workers have used this solvent to good advantage in previous enzyme histochemical techniques, acetone could well be tried more generally for work in tissue culture techniques and in other enzyme histochemical studies.

Another advantage of rapid tissue dehydration, not particularly because of the solvent employed, is the retention of easily loosened and obliterated delicate structures gained by the minimum amount of handling of the material.

By employing several diverse types of plant materials that continue to be the subjects of further expanded studies, the rapid tissue dehydration method has been found suitable under the conditions described and should be applicable for most microtechnique tissue processes.

REFERENCES

* Received April 17, 1956, from Duquesne University, School of Pharmacy, Pittsburgh 19, Pa.

The writer wishes to express grateful appreciation for the earlier reviews of this report by the late Dr. Hugh C. Muldoon, Dean Emeritus, Duquesne University School of Pharmacy, and by Dr. Franklin A. Neva, Department of Tropical Public Health, Harvard School of Public Health, Boston, Mass.

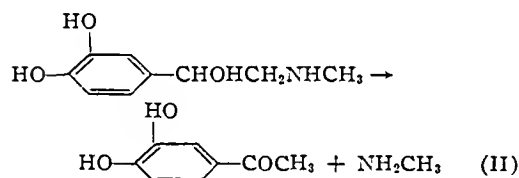
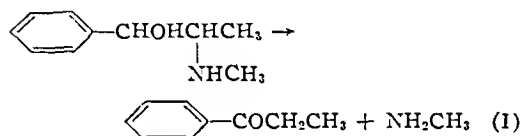
- (1) Bush, V., *Science*, 115, 649 (1952).
 - (2) Bush, V., *ibid.*, 122, 119 (1955).
 - (3) Lee, C., *Stain Technol.*, 28, 285 (1953).
 - (4) Gomori, G., *Proc. Soc. Exptl. Biol. Med.*, 68, 354 (1948).
 - (5) Lars, A., *Science*, 107, 278 (1948).
 - (6) Saiki, A., and Kling, R., *ibid.*, 109, 419 (1949).
- ¹ W. A. Hammond Drierite Company's anhydrous calcium sulfate.

A Note on the Detection of Ephedrine by Spot Test*

By FRITZ FEIGL and ERNESTO SILVA

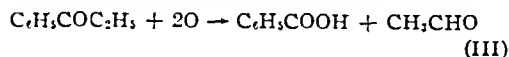
SPOT TEST METHODS are not only just as reliable as the macromethods given in pharmacopeias for the identification of medicinal and pharmaceutical preparations, but also possess the great advantage of saving material, time, and effort. This fact has been mentioned previously (1) and methods for identifying certain compounds which had not previously been noted in the pharmacopeias were presented. As a supplement to this work, a new and quite specific test for ephedrine is described below, which is of interest in the testing of pressor substances (sympathomimetic amines).

Since alkali easily causes an hydramine cleavage of ephedrine and adrenaline according to the equations:



Tests for these two amines have been described (2), which are based on the detection of the mono-methylamine produced. The limits of detection by the spot test technique are 5 γ of ephedrine and 8 γ of adrenaline. When ephedrine alone is to be detected by the hydramine cleavage, the preliminary removal of adrenaline is necessary. This is easy to achieve, since ephedrine is very soluble in ether, while adrenaline is completely insoluble. In dealing with acid solutions of chlorides, sulfates, etc., one must, therefore, shake first with alkali and then with ether.

It has been found that the hydramine cleavage of ephedrine, can be detected by making use of its behavior on pyrolysis in the presence of oxidizing agents. If ephedrine hydrochloride is evaporated to dryness in the presence of alkali and the dry residue heated over an open flame after being mixed with MnO₂, PbO₂, Pb₂O₄, Mn₂O₃, or Co₂O₃, acetaldehyde is split out. This is apparently the result of the oxidation of the phenylethyl ketone produced by the hydramine cleavage, according to the reaction:



Schmidt (5) has found that phenylethyl ketone is split out on dry distillation of chlorides of ephedrine and pseudo-ephedrine in a stream of CO₂. In accord with this, and with the oxidation according to (III), is the fact that ephedrine mixed only with excess CaO or MnO₂ and heated, gives off acetaldehyde, although only in small quantities. The acetaldehyde formed by this pyrolytic oxidation can be identified in the gas phase by the test of Lewin (4) (blue color formation in a piperidine-sodium nitroprusside solution).

Dry heating of ephedrine or its salts with sodium bismuthate (NaBiO₃) has proved most suitable for performing the decomposition to acetaldehyde and for using it analytically. This basic compound of pentavalent bismuth acts simultaneously as an alkali to catalyze the hydramine cleavage and as an oxidizing agent for the phenylethyl ketone produced. The acetaldehyde is detected with a morpholine-sodium nitroprusside solution (3).

Adrenaline in centigram quantities does not produce any acetaldehyde, and hence it may be distinguished from ephedrine absolutely.

Procedure.—A minimal amount of the solid substance to be tested, or of the dry residue from a drop of a solution of the base or its salts is mixed with several centigrams of sodium bismuthate in a micro test tube. A piece of filter paper which has been moistened with a drop of the aldehyde reagent is placed over the open end of the tube, and the bottom is heated with a microflame. A positive test is shown by the formation of a circular blue spot on the yellow reagent paper. The intensity of the color depends on the quantity of ephedrine.

When ephedrine is to be detected in very dilute solution it is best to put some glass powder into the tube, and to evaporate a drop of the test solution on this powder, after which the sodium bismuthate is added and mixed in. In this way one obtains good contact for the pyrolytic oxidation. Limit of detection: 5 γ ephedrine.

Reagent.—A freshly prepared mixture of equal volumes of 20% water solution of morpholine and 5% solution of sodium nitroprusside in water was used.

It may be expected that Ephictonal (*p*-amino compound of ephedrine) will equally split off acetaldehyde on pyrolytic oxidation.

The user of the test described above should note that compounds with —OC₂H₅ and >NC₂H₅ groups also yield acetaldehyde on dry heating with sodium bismuthate, but in smaller amounts than does ephedrine.

REFERENCES

* Received September 10, 1957, from the Laboratorio da Producao Mineral, Ministerio da Agricultura, Rio de Janeiro, Brazil.

Translated by Peter Oesper, Hahnemann Medical College, Phila., Pa.

(1) Feigl, F., and Silva, E., *Drug Standards*, 23, 114 (1955); see also Feigl, F., "Spot Tests in Organic Analysis," 5th ed., New York, 1956, Chap. VI.

(2) Feigl, F., and Feigl, H. E., *Helv. Chim. Acta*, 38, 459 (1955).

(3) Feigl, F., "Spot Tests in Organic Analysis," 5th ed., New York, 1956, p. 334.

(4) Lewin, L., *Ber.*, 32, 3388 (1899).

(5) Schmidt, E., *Arch. Pharm.* 247, 141 (1909).

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Synthesis and Investigation of Certain Biological Activities of Some Thiophosphoric Acid Derivatives*

By MITCHELL L. BORKE† and ERNST R. KIRCH

Two series of N-substituted phosphoroamidothioates and two phosphorochloridothioates were synthesized. Of these, eleven phosphoroamidothioates have not been prepared previously. The compounds were investigated for their activity *in vitro* toward cholinesterase and for their ability to inhibit the growth of certain microorganisms as well as the germination of some plant species. Only the two phosphorochloridothioates showed anticholinesterase activity. None of the synthesized compounds inhibited the growth of the microorganisms selected for this investigation or retarded the growth of roots and shoots of oats or black mustard. The results of the experiments were correlated with the physical properties, the steric configuration and the electronic structure of the investigated compounds.

DURING RECENT YEARS certain organophosphorus compounds have come into use as insecticides and fungicides, while others are classified as "nerve gases."

It was in the middle thirties that Schrader (1) began searching for new synthetic insecticides as substitutes for nicotine, rotenone, and pyrethrin which had to be imported into Germany, and turned his attention to certain organophosphorus compounds.

During the screening tests for insecticidal properties Gross (1) noticed the anticholinesterase activity and the pharmacological action on warm-blooded animals of a number of organophosphorus compounds.

* Received November 12, 1957, from the Department of Chemistry, College of Pharmacy, University of Illinois Chicago 12.

Abstracted in part from a thesis submitted to the Graduate College of the University of Illinois by Mitchell L. Borke in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

† Present address: School of Pharmacy, Duquesne University, Pittsburgh 19, Pa.

Shortly after the outbreak of World War II, teams were organized in Great Britain and charged with the development of new chemical warfare agents. Several of these groups concentrated on organophosphorus compounds containing fluorine (2). Because certain derivatives looked promising as war gases, improved methods of their preparation adaptable to a large scale production were worked out (3).

Since the end of the war numerous reports concerning the organophosphorus compounds have appeared in the literature. The investigations have been related mainly to the biological activity of some of these substances (4-6) and to the development of effective antidotes against those organophosphorus compounds which could be used as "nerve gases" (7-11).

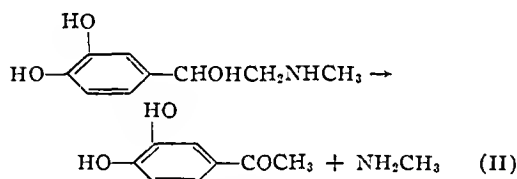
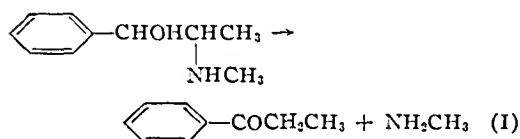
One of the phases of the research related to the biological activity of the phosphorus containing organic compounds has been the work connected

A Note on the Detection of Ephedrine by Spot Test*

By FRITZ FEIGL and ERNESTO SILVA

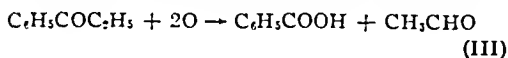
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REFERENCES

- (1) Feigl, F., and Silva, E., *Drug Standards*, 23, 111 (1955); see also Feigl, F., "Spot Tests in Organic Analysis," 5th ed., New York, 1956, Chap. VI.
- (2) Feigl, F., and Feigl, H. E., *Helv. Chim. Acta*, 38, 459(1955).
- (3) Feigl, F., "Spot Tests in Organic Analysis," 5th ed., New York, 1956, p. 331.
- (4) Lewin, L., *Ber.*, 32, 3388(1899).
- (5) Schmidt, E., *Arch. Pharm.* 247, 141(1909).

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Synthesis and Investigation of Certain Biological Activities of Some Thiophosphoric Acid Derivatives*

By MITCHELL L. BORKE† and ERNST R. KIRCH

Two series of N-substituted phosphoroamidothioates and two phosphorochloridothioates were synthesized. Of these, eleven phosphoroamidothioates have not been prepared previously. The compounds were investigated for their activity *in vitro* toward cholinesterase and for their ability to inhibit the growth of certain microorganisms as well as the germination of some plant species. Only the two phosphorochloridothioates showed anticholinesterase activity. None of the synthesized compounds inhibited the growth of the microorganisms selected for this investigation or retarded the growth of roots and shoots of oats or black mustard. The results of the experiments were correlated with the physical properties, the steric configuration and the electronic structure of the investigated compounds.

DURING RECENT YEARS certain organophosphorus compounds have come into use as insecticides and fungicides, while others are classified as "nerve gases."

It was in the middle thirties that Schrader (1) began searching for new synthetic insecticides as substitutes for nicotine, rotenone, and pyrethrin which had to be imported into Germany, and turned his attention to certain organophosphorus compounds.

During the screening tests for insecticidal properties Gross (1) noticed the anticholinesterase activity and the pharmacological action on warm-blooded animals of a number of organophosphorus compounds.

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Abstracted in part from a thesis submitted to the Graduate College of the University of Illinois by Mitchell L. Borke in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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Shortly after the outbreak of World War II, teams were organized in Great Britain and charged with the development of new chemical warfare agents. Several of these groups concentrated on organophosphorus compounds containing fluorine (2). Because certain derivatives looked promising as war gases, improved methods of their preparation adaptable to a large scale production were worked out (3).

Since the end of the war numerous reports concerning the organophosphorus compounds have appeared in the literature. The investigations have been related mainly to the biological activity of some of these substances (4-6) and to the development of effective antidotes against those organophosphorus compounds which could be used as "nerve gases" (7-11).

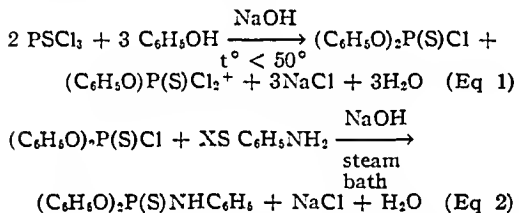
One of the phases of the research related to the biological activity of the phosphorus containing organic compounds has been the work connected

with the structure activity relationship (SAR) *in vitro* as well as *in vivo* (12, 13)

In this investigation two series of N-substituted phosphoroamidothioates and two phosphorochloridothioates presented in Table I were synthesized. In order to determine the structure activity relationship of these two groups of organophosphorus compounds, they were tested for their activity toward acetylcholinesterase (AChase), for their action on certain microorganisms and for their influence on the germination of certain plant species

EXPERIMENTAL

* The preparation of the N substituted diphenyl and di (*p* tolyl) phosphoroamidothioates was achieved by a two step synthesis similar to that reported by Autenrieth and Hildebrand (14) for the synthesis of diphenyl and di (*p* tolyl)-phosphoroamidothioates and which can be represented by the following equations



Procedure.—Two moles (239 Gm) of thiophosphoryl chloride were added in one portion to a well-cooled solution of 3 moles (282 Gm) of phenol in an excess of 10% sodium hydroxide and the mixture stirred for four hours. Cooling was continued during this period so that the temperature did not rise above 50°. Since the reaction mixture should be alkaline at all times, additional sodium hydroxide solution was added when necessary.

At the end of the four hour period, the mixture was extracted with several portions of ether, and the combined ether extracts washed with 10% sodium hydroxide solution, then with water, and finally dried over anhydrous sodium sulfate. The solvent was distilled off and the residue subjected to vacuum distillation in order to remove any unreacted thiophosphoryl chloride and phenyl phosphorodichloridothioate. The distillation was stopped at 132°/11 mm Hg.

The diphenyl phosphorochloridothioate remaining in the distilling flask solidified upon cooling and stirring. It was recrystallized from hot ethanol. Melting point found 66–68° (uncorrected).

Di (*p* tolyl) phosphorochloridothioate was prepared in a similar way and was recrystallized from hot ethanol. Melting point found 49–51° (uncorrected).

The N-substituted diphenyl and di (*p* tolyl) phosphoroamidothioates were prepared by adding a calculated amount of the phosphorochloridothioate to an excess of the particular amine in a 10% sodium hydroxide solution. The ratio of the phosphorochloridothioate to the amine was 1:4. All amines

TABLE I

No	R ₁	R ₂	Yield %	M p °C *	Nitrogen %		Phosphorus %		Sulfur %	
					Calcd	Found	Calcd	Found	Calcd	Found
1 ^a	Cl—	Cl—	33	64–66	—	—	10.89	10.93	11.24	10.93
2 ^a	C ₆ H ₅ NH—	C ₆ H ₅ NH—	25	90–92	4.10	4.55	9.08	9.11	9.39	9.42
3 ^a	<i>o</i> CH ₃ C ₆ H ₄ NH—	<i>o</i> CH ₃ C ₆ H ₄ NH—	17	68–70	3.94	3.96	8.72	8.86	9.02	9.00
4 ^a	<i>m</i> CH ₃ C ₆ H ₄ NH—	<i>m</i> CH ₃ C ₆ H ₄ NH—	45	86–88	3.94	3.91	8.72	8.80	9.02	9.05
5 ^a	<i>p</i> CH ₃ C ₆ H ₄ NH—	<i>p</i> CH ₃ C ₆ H ₄ NH—	69	94–96	3.94	3.94	8.72	8.82	9.02	9.02
6 ^a	<i>o</i> CH ₃ OC ₆ H ₄ NH—	<i>o</i> CH ₃ OC ₆ H ₄ NH—	39	84–86	3.77	3.78	8.35	8.48	8.63	8.66
7 ^a	<i>p</i> CH ₃ OC ₆ H ₄ NH—	<i>p</i> CH ₃ OC ₆ H ₄ NH—	25	83–85	3.77	3.78	8.35	8.43	8.63	8.64
8 ^a	CH ₃ (CH ₂) ₄ N—	CH ₃ (CH ₂) ₄ N—	50	49–51	4.20	4.16	9.30	9.42	9.60	9.64
9 ^a	Cl—	Cl—	quant	49–51	—	—	9.92	9.66	10.23	10.22
10 ^b	C ₆ H ₅ NH—	C ₆ H ₅ NH—	41	103–105	3.73	3.70	8.26	8.36	8.54	8.41
11 ^b	<i>o</i> CH ₃ C ₆ H ₄ NH—	<i>o</i> CH ₃ C ₆ H ₄ NH—	21	93–95	3.65	3.67	8.08	8.20	8.36	8.45
12 ^b	<i>m</i> CH ₃ C ₆ H ₄ NH—	<i>m</i> CH ₃ C ₆ H ₄ NH—	17	93–95	3.65	3.66	8.08	8.02	8.36	8.36
13 ^b	<i>p</i> CH ₃ C ₆ H ₄ NH—	<i>p</i> CH ₃ C ₆ H ₄ NH—	33	94–96	3.65	3.68	8.08	8.19	8.36	8.32
14 ^b	<i>o</i> CH ₃ OC ₆ H ₄ NH—	<i>o</i> CH ₃ OC ₆ H ₄ NH—	17	77–79	3.51	3.54	7.76	7.83	8.03	8.08
15 ^b	<i>p</i> CH ₃ OC ₆ H ₄ NH—	<i>p</i> CH ₃ OC ₆ H ₄ NH—	28	58–60	3.51	3.46	7.76	7.78	8.03	8.00

* All melting points are uncorrected. ^a Compounds 1, 2, 9 and 10 have been reported in the literature (14).

(Eastman Kodak Co.) were purified by distillation prior to their use. The mixture was heated on a steam bath for several hours while stirred mechanically. During this time samples of the mixture were withdrawn from the reaction vessel and tested with 0.1 *N* silver nitrate solution for the presence of chloride ion. When large amounts of it could be detected in the mixture as indicated by a heavy precipitate of silver chloride, the reaction was stopped by cooling to room temperature. The organic components were extracted with several portions of ether and the combined ether extracts washed with several portions of dilute hydrochloric acid, then with water, and finally dried over anhydrous sodium sulfate.

The solvent was removed by distillation using a steam bath, the residue cooled in an ice bath to promote crystallization, and the crude phosphoroamidothioates recrystallized from hot ethanol. In several cases it was found advisable to recrystallize the compound from isoamyl alcohol. All of the phosphoroamidothioates were white crystalline solids, extremely insoluble in water but soluble in common organic solvents.

The compounds were analyzed gravimetrically for sulfur and phosphorus (15, 16). Their nitrogen content was determined by a semimicro Kjeldahl method (17).

Investigation of Biological Activity.—All of the fifteen compounds were tested *in vitro* for their activity toward acetylcholinesterase (AChase) by the use of the Hestrin colorimetric method (18).

Materials and Equipment.—*Phosphate buffer*, 0.134 *M*, pH 7.2, was prepared by mixing 7 parts by volume of a solution of 23.8 Gm. of disodium hydrogen phosphate dodecahydrate (Mallinckrodt Chem. Works) per liter and 3 parts of a solution of 18.2 Gm. of potassium dihydrogen phosphate (Merck & Co., Inc., Reagent) per liter, pH being adjusted to 7.2 if necessary.

Acetylcholine, 0.04 *M*, 0.9045 Gm. of acetylcholine bromide (Eastman Kodak No. 2117) in 100 ml. of 0.001 *M* acetate buffer, pH 4.5.

Acetylcholinesterase (Winthrop Laboratories), 20,000 units.

Hydroxylamine hydrochloride, 2 *M*, 69.5 Gm. (Eastman Kodak No. 340) dissolved in distilled water to 500 ml.

The above solutions as well as the acetylcholinesterase preparation were stored in the refrigerator.

Sodium hydroxide, 3.5 *M*, 70 Gm. of sodium hydroxide pellets (Mallinckrodt Chem. Works, U. S. P. Grade) dissolved in distilled water to 500 ml.

Alkaline hydroxylamine, equal volumes of 2 *M* hydroxylamine hydrochloride and 3.5 *M* sodium hydroxide solutions mixed shortly before use. The solution was made up freshly for each set of samples run.

Hydrochloric acid, concentrated acid (sp.gr. 1.18) diluted with 2 volumes of distilled water.

Ferric chloride, 0.37 *M*, 50 Gm. of ferric chloride hexahydrate (Baker & Adamson, Reagent) dissolved in 0.1 *M* hydrochloric acid to 500 ml.

Potassium chloride, 0.3 *M*, 22.4 Gm. of potassium chloride (Baker & Adamson, Reagent) dissolved in 1 liter of distilled water.

Trichloroacetic acid, 50%, prepared by dissolving 50 Gm. of trichloroacetic acid (Merck & Co., Inc., U. S. P. Grade) in 100 ml. of distilled water.

In the experiments designed to investigate the *in vitro* AChase inhibitory activity of the synthesized compounds, the substrate was prepared by diluting one part of the 0.04 *M* acetylcholine bromide stock solution with eight parts of the phosphate buffer (pH 7.2). One ml. of this solution provided 4.44 micromoles of acetylcholine bromide, and this concentration of the substrate was within the range in which the Hestrin method gives reproducible results.

It was found experimentally that 0.444 micromoles of acetylcholine bromide per ml. of the final solution provided a suitable concentration of the substrate so that both in the absence of the enzyme and in its presence the color developed obeyed the Beer-Lambert Law.

Stable AChase preparations were made by the adsorption of 0.05-ml. aliquots of a 1% enzyme solution in a stabilizing medium on Whatman No. 1 filter paper disks and storing in a refrigerator over anhydrous CaCl_2 . This procedure described by Fleisher, *et al.* (19), supplied a concentration of the enzyme suitable for the determinations carried out in this investigation, when one paper disk was eluted with 17 ml. of 0.3 *M* potassium chloride solution in a refrigerator for seventy-five minutes, and when 2 ml. of this eluate per 1 ml. of the 0.00444 *M* acetylcholine bromide solution were used in the assay.

These amounts also provided the optimum ratio of the substrate to enzyme so that the velocity of the AChase catalyzed hydrolysis of acetylcholine was at its maximum as seen in Fig. 1.

All determinations designed to investigate the *in vitro* AChase activity of the synthesized compounds were run in duplicates according to the setup presented in Table II.

TABLE II.—SETUP FOR ACHASE ACTIVITY DETERMINATIONS

	Controls			Concn in mcg / ml				
	No Enzyme	Color Interference ^a	Enzyme Activity	250	50	25	10	1
1 Ethanol (vehicle)	0 2 ml.	0 0 ml.	0 2 ml.	0 0	0 0	0 0	0 0	0 0
2 Compound in ethanol	0 0	0 2	0 0	0 2	0 2	0 2	0 2	0 2
3 Enzyme eluate	0 0	0 0	2 0	2 0	2 0	2 0	2 0	2 0
4 0.3 <i>M</i> KCl	2 0	2 0	0 0	0 0	0 0	0 0	0 0	0 0
5 0.00444 <i>M</i> acetylcholine bromide	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0

^a This control was introduced to provide a check of the enzyme activity in the test tubes containing the compounds in 250 mcg / ml concentration because some of them caused turbidity in the presence of the other reagents

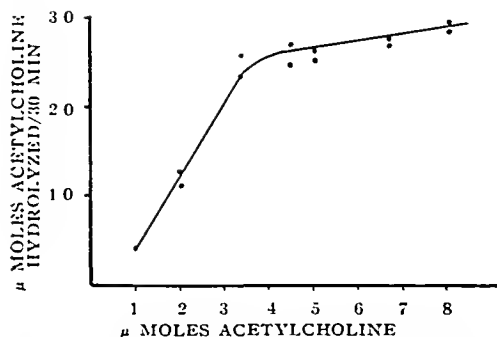


Fig. 1.—Determination of optimum substrate-enzyme concentrations.

Substances 1 through 4 in Table II were placed in the test tubes in the indicated order and incubated in a thermostatically controlled water bath at 37° for thirty minutes. Then 1 ml. of the substrate (reagent 5) was added to each test tube and the incubation continued for another thirty minutes. At the end of the incubation period, two drops of 50% trichloroacetic acid were added to each test tube to inactivate the enzyme. The tubes were removed from the water bath, the color developed using successively 2 ml. of alkaline hydroxylamine, 1 ml. of hydrochloric acid (1:2), and 1 ml. of 0.37 *M* ferric chloride solution. The tubes were shaken thoroughly after the addition of each reagent to prevent the formation of gas bubbles. Distilled water was added to each test tube to bring the volume to 10 ml., and the color read in a suitable instrument¹ at 540 *mμ* after thirty minutes.

Of the fifteen compounds tested *in vitro* for their activity toward AChase only diphenyl phosphorochloridothioate and di-(*p*-tolyl) phosphorochloridothioate inhibited the enzyme, and their *I*₅₀² were 2.30×10^{-4} *M* and 2.60×10^{-4} *M* respectively. None of the *N*-substituted phosphoramidothioates showed any inhibition of AChase under the conditions of the experiments.

In order to determine the type of AChase inhibition demonstrated by the two phosphorochloridothioates, it was decided to use the method reported by Aekermann and Potter (20). In this procedure, variable amounts of the enzyme plus or minus the inhibitor are plotted against the rate of hydrolysis of the substrate. A straight line results for any given concentration of the inhibitor and when no inhibitor is present. In the latter case the line crosses the origin. When an enzyme is inhibited reversibly, the line also crosses the origin but its slope is smaller than that of the control. If, however, the inhibition is irreversible or pseudo-irreversible, the slope of the line is the same or nearly the same as that of the control but it passes the abscissa to the right of the origin and at a distance which is proportional to the amount of the inhibitor

The examination of Figs. 2 and 3 representing the plots of the rate of hydrolysis of the substrate against varied amounts of the enzyme eluate for 50, 100, and 250 mcg./ml. of diphenyl and di-(*p*-tolyl) phosphorochloridothioate indicates that both compounds inhibit AChase irreversibly or pseudoirreversibly.

The biological activity of the described *N*-substituted phosphoramidothioates as well as that of the two phosphorochloridothioates toward living organisms was investigated by testing their action on the multiplication of certain bacteria and fungi, and their influence on the germination of the seeds of some plants.

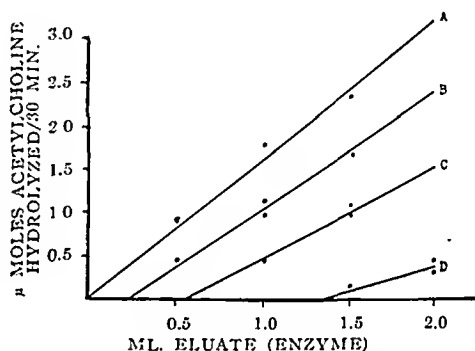


Fig. 2.—Analysis of AChase inhibition by diphenyl phosphorochloridothioate according to the procedure of Aekermann and Potter (20). *A*—controls; *B*—inhibitor present at 5 mcg./ml. final concentration; *C*—inhibitor present at 10 mcg./ml. final concentration; *D*—inhibitor present at 25 mcg./ml. final concentration.

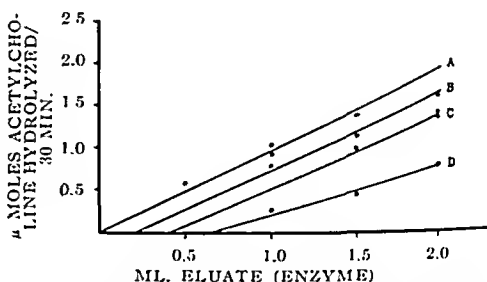


Fig. 3.—Analysis of AChase inhibition by di-(*p*-tolyl) phosphorochloridothioate according to the procedure of Aekermann and Potter (20). *A*—controls; *B*—inhibitor present at 5 mcg./ml. final concentration; *C*—inhibitor present at 10 mcg./ml. final concentration; *D*—inhibitor present at 25 mcg./ml. final concentration.

A report was published about the inhibition of growth of *Aspergillus niger* and *Aspergillus flavus* by certain organophosphorus compounds (21). Thus the synthesized thiophosphates were investi-

¹ Coleman Universal Spectrophotometer, Model 11, was used in this investigation.

² *I*₅₀ is the molar concentration of an inhibitor required to inhibit 50% of the enzyme.

gated for their activity toward microorganisms. *Micrococcus pyogenes* var. *aureus*, *Escherichia coli* and *Candida albicans* were used as representatives of the Gram-positive and Gram-negative bacteria and the fungi respectively. The agar cup method and the paper disk technique were chosen for this investigation, and three concentrations of the compounds (1:10, 1:100, and 1:1000) were used in the experiments.

For the agar cup method the compounds were dissolved in extra heavy mineral oil (Nujol, Plough, Inc.) with the aid of slight heat. Due to the poor solubility of the compounds, all of the 1:10 solutions yielded uniform heavy suspensions upon cooling to the incubation temperature (37°) and were used as such to fill the agar cups. A control was provided by filling one of the agar cups with Nujol.

In the paper disk method the compounds were dissolved in chloroform (Mallinckrodt Chemical Works, Analytical Reagent) to yield 1:10, 1:100, and 1:1000 solutions (w/v). The disks, 11 mm. in diameter and prepared from E. & D. No. 615 filter paper, were immersed in the corresponding solutions for a few seconds and the solvent permitted to evaporate in the air. The disks were placed upon agar seeded with the appropriate microorganisms and incubated at 37°. A control was provided by a disk immersed in the solvent and treated as above.

In both methods nutrient agar was used for *Micrococcus pyogenes* and *Escherichia coli* and Sabaroud's agar for *Candida albicans*. The plates were read at twenty-four hours and forty-eight hours. No inhibition of growth by either the N-substituted phosphoroamidothioates or by the two phosphorochloridothioates which inhibited AChase, was observed.

The effect of dialkyl anilidophosphates on the germination of the oat (*Avena sativa*, L.) and black mustard seeds (*Brassica nigra*, L.) as representing the mono- and dicotyledonous plant species was studied by Ramaswami, *et al.* (22). These authors found some inhibition of the germination of oats but no effect on the germination of black mustard seeds. Because of the structural similarity between the compounds tested by Ramaswami and those described in this work, it was decided to investigate the influence of the two AChase inhibitors and two N-substituted phosphoroamidothioates (non-inhibitors) chosen at random on the germination of oat and black mustard seeds.

The following procedure, a slight modification of that described by Ramaswami, *et al.*, was used in the germination experiments. A 0.5-ml. aliquot of the ethanolic solution of the compound corresponding to 125 mcg. of it was deposited in the center of a filter paper disk 12.5 cm. in diameter (Whatman No. 1). The solvent was permitted to evaporate in the air and the filter paper placed on a 5-inch watch glass containing 25 ml. of distilled water. The edge of the paper came flush with the rim of the watch glass. The paper was pressed down gently with a piece of glass rod to touch the water in the watch glass, and the moist area allowed to spread evenly throughout the paper. Thus the concentrations of the compounds tested ranged from 1.25 to 1.76 moles in one million milliliters depending upon the molecular weight of the particular compound.

Ten seeds selected on the basis of their physical appearance were placed around and within 0.5 cm.

from the rim of the watch glass. This setup affords a convenient means of providing a moist paper surface upon which the seeds can be placed without letting them be immersed in the solution. A control was provided using 0.5 ml. of ethanol and treating it in the above described manner. The observations lasted four days and no effect on the germination of either the oat or the black mustard seeds was noticed.

DISCUSSION

Of the fifteen compounds tested for cholinesterase inhibition *in vitro*, only diphenyl phosphorochloridothioate and di-(*p*-tolyl) phosphorochloridothioate were shown to be active against this enzyme.

The general method for comparing inhibitors consists of the determination of the molar concentration of the compounds required to inhibit 50% of the enzyme (I_{50}). The I_{50} value was calculated to be 2.30×10^{-4} M for diphenyl phosphorochloridothioate and 2.60×10^{-4} M for di-(*p*-tolyl) phosphorochloridothioate.

The results of the experiments performed to determine the *in vitro* biological activity of the compounds used in this investigation toward AChase can be explained on the basis of the electronic theory. It is generally accepted that during a reaction between an alkyl phosphate and cholinesterase the enzyme becomes phosphorylated (12, 23). There is also evidence that the phosphate is split in this reaction (12, 24).

The phosphorylation of the enzyme can be rationalized to proceed in a stepwise manner as represented in Fig. 4.

It is evident that the success of the first step in the phosphorylation reaction depends upon the electrophilic character of the phosphorus atom. In diphenyl phosphorochloridothioate and di-(*p*-tolyl) phosphorochloridothioate it is influenced primarily by the chlorine atom, which polarizes the P—Cl bond by the inductive effect ($P \rightarrow Cl$).

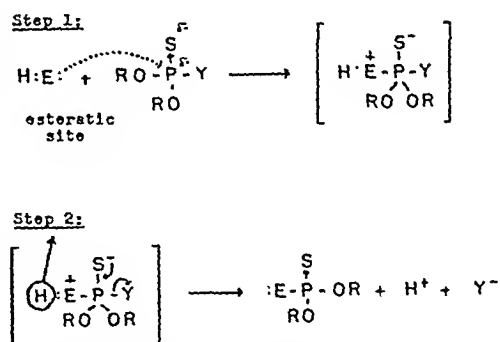


Fig. 4.—Electronic mechanism for the phosphorylation of AChase by an alkyl phosphate type inhibitor. I^- —strongly electronegative group or atom.

Since the determined I_{50} values for the two phosphorochloridothioates are very close to each other, it can be concluded that the presence of a methyl group in di-(*p*-tolyl) phosphorochloridothioate does not influence the electrophilic character of the phosphorus atom to any significant degree. Further-

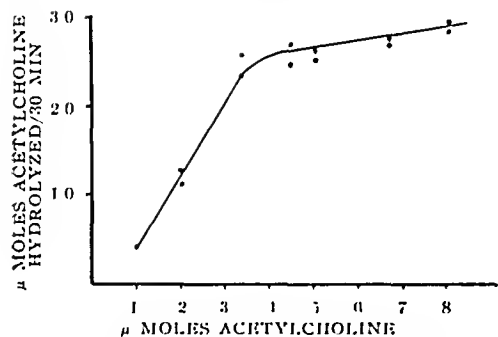


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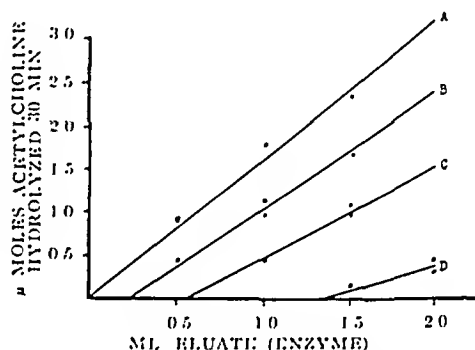


Fig. 2—Analysis of AChase inhibition by diphenyl phosphorochloridothioate according to the procedure of Ackermann and Potter (20). A—controls; B—inhibitor present at 5 *mcg./ml.* final concentration; C—inhibitor present at 10 *mcg./ml.* final concentration; D—inhibitor present at 25 *mcg./ml.* final concentration.

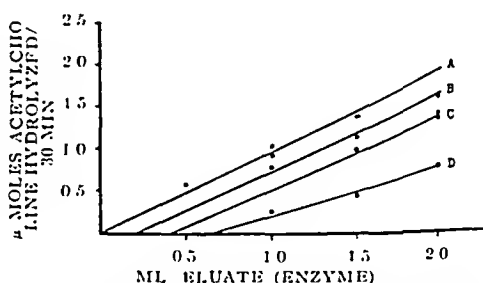


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The following procedure, a slight modification of that described by Ramaswami, *et al.*, was used in the germination experiments. A 0.5-ml. aliquot of the ethanolic solution of the compound corresponding to 125 mcg. of it was deposited in the center of a filter paper disk 12.5 cm. in diameter (Whatman No. 1). The solvent was permitted to evaporate in the air and the filter paper placed on a 5-inch watch glass containing 25 ml. of distilled water. The edge of the paper came flush with the rim of the watch glass. The paper was pressed down gently with a piece of glass rod to touch the water in the watch glass, and the moist area allowed to spread evenly throughout the paper. Thus the concentrations of the compounds tested ranged from 1.25 to 1.76 moles in one million milliliters depending upon the molecular weight of the particular compound.

Ten seeds selected on the basis of their physical appearance were placed around and within 0.5 cm.

from the rim of the watch glass. This setup affords a convenient means of providing a moist paper surface upon which the seeds can be placed without letting them be immersed in the solution. A control was provided using 0.5 ml. of ethanol and treating it in the above described manner. The observations lasted four days and no effect on the germination of either the oat or the black mustard seeds was noticed.

DISCUSSION

Of the fifteen compounds tested for cholinesterase inhibition *in vitro*, only diphenyl phosphorochloridothioate and di-(*p*-tolyl) phosphorochloridothioate were shown to be active against this enzyme.

The general method for comparing inhibitors consists of the determination of the molar concentration of the compounds required to inhibit 50% of the enzyme (I_{50}). The I_{50} value was calculated to be 2.30×10^{-4} M for diphenyl phosphorochloridothioate and 2.60×10^{-4} M for di-(*p*-tolyl) phosphorochloridothioate.

The results of the experiments performed to determine the *in vitro* biological activity of the compounds used in this investigation toward AChase can be explained on the basis of the electronic theory. It is generally accepted that during a reaction between an alkyl phosphate and cholinesterase the enzyme becomes phosphorylated (12, 23). There is also evidence that the phosphate is split in this reaction (12, 24).

The phosphorylation of the enzyme can be rationalized to proceed in a stepwise manner as represented in Fig. 4.

It is evident that the success of the first step in the phosphorylation reaction depends upon the electrophilic character of the phosphorus atom. In diphenyl phosphorochloridothioate and di-(*p*-tolyl) phosphorochloridothioate it is influenced primarily by the chlorine atom, which polarizes the P—Cl bond by the inductive effect ($P \rightarrow Cl$).

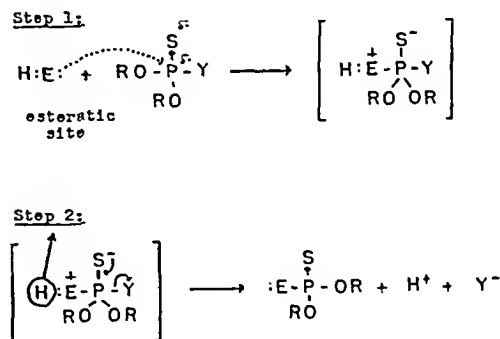


Fig. 4.—Electronic mechanism for the phosphorylation of AChase by an alkyl phosphate type inhibitor. I⁻—strongly electronegative group or atom.

Since the determined I_{50} values for the two phosphorochloridothioates are very close to each other, it can be concluded that the presence of a methyl group in di-(*p*-tolyl) phosphorochloridothioate does not influence the electrophilic character of the phosphorus atom to any significant degree. Further-

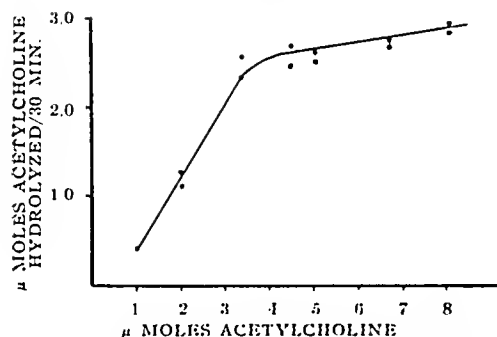


Fig. 1.—Determination of optimum substrate-enzyme concentrations.

Substances 1 through 4 in Table II were placed in the test tubes in the indicated order and incubated in a thermostatically controlled water bath at 37° for thirty minutes. Then 1 ml. of the substrate (reagent 5) was added to each test tube and the incubation continued for another thirty minutes. At the end of the incubation period, two drops of 50% trichloroacetic acid were added to each test tube to inactivate the enzyme. The tubes were removed from the water bath, the color developed using successively 2 ml. of alkaline hydroxylamine, 1 ml. of hydrochloric acid (1:2), and 1 ml. of 0.37 *M* ferric chloride solution. The tubes were shaken thoroughly after the addition of each reagent to prevent the formation of gas bubbles. Distilled water was added to each test tube to bring the volume to 10 ml., and the color read in a suitable instrument¹ at 540 mμ after thirty minutes.

Of the fifteen compounds tested *in vitro* for their activity toward AChase only diphenyl phosphorochloridothioate and di-(*p*-tolyl) phosphorochloridothioate inhibited the enzyme, and their I_{50}^2 were 2.30×10^{-4} *M* and 2.60×10^{-4} *M* respectively. None of the *N*-substituted phosphoroamidothioates showed any inhibition of AChase under the conditions of the experiments.

In order to determine the type of AChase inhibition demonstrated by the two phosphorochloridothioates, it was decided to use the method reported by Ackermann and Potter (20). In this procedure, variable amounts of the enzyme plus or minus the inhibitor are plotted against the rate of hydrolysis of the substrate. A straight line results for any given concentration of the inhibitor and when no inhibitor is present. In the latter case the line crosses the origin. When an enzyme is inhibited reversibly, the line also crosses the origin but its slope is smaller than that of the control. If, however, the inhibition is irreversible or pseudo-irreversible, the slope of the line is the same or nearly the same as that of the control but it passes the abscissa to the right of the origin and at a distance which is proportional to the amount of the inhibitor.

The examination of Figs. 2 and 3 representing the plots of the rate of hydrolysis of the substrate against varied amounts of the enzyme eluate for 50, 100, and 250 mcg./ml. of diphenyl and di-(*p*-tolyl) phosphorochloridothioate indicates that both compounds inhibit AChase irreversibly or pseudoirreversibly.

The biological activity of the described *N*-substituted phosphoroamidothioates as well as that of the two phosphorochloridothioates toward living organisms was investigated by testing their action on the multiplication of certain bacteria and fungi, and their influence on the germination of the seeds of some plants.

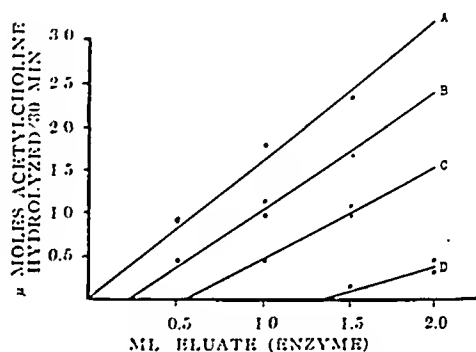


Fig. 2.—Analysis of AChase inhibition by diphenyl phosphorochloridothioate according to the procedure of Ackermann and Potter (20). A—controls; B—inhibitor present at 5 mcg./ml. final concentration; C—inhibitor present at 10 mcg./ml. final concentration; D—inhibitor present at 25 mcg./ml. final concentration.

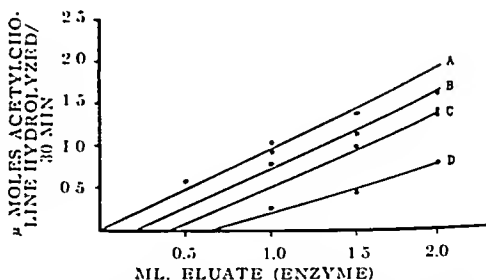


Fig. 3.—Analysis of AChase inhibition by di-(*p*-tolyl) phosphorochloridothioate according to the procedure of Ackermann and Potter (20). A—controls; B—inhibitor present at 5 mcg./ml. final concentration; C—inhibitor present at 10 mcg./ml. final concentration; D—inhibitor present at 25 mcg./ml. final concentration.

¹ Coleman Universal Spectrophotometer, Model 11, was used in this investigation.

² I_{50} is the molar concentration of an inhibitor required to inhibit 50% of the enzyme.

A report was published about the inhibition of growth of *Aspergillus niger* and *Aspergillus flavus* by certain organophosphorus compounds (21). Thus the synthesized thiophosphates were investi-

gated for their activity toward microorganisms. *Micrococcus pyogenes* var. *aureus*, *Escherichia coli* and *Candida albicans* were used as representatives of the Gram-positive and Gram-negative bacteria and the fungi respectively. The agar cup method and the paper disk technique were chosen for this investigation, and three concentrations of the compounds (1:10, 1:100, and 1:1000) were used in the experiments.

For the agar cup method the compounds were dissolved in extra heavy mineral oil (Nujol, Plough, Inc.) with the aid of slight heat. Due to the poor solubility of the compounds, all of the 1:10 solutions yielded uniform heavy suspensions upon cooling to the incubation temperature (37°) and were used as such to fill the agar cups. A control was provided by filling one of the agar cups with Nujol.

In the paper disk method the compounds were dissolved in chloroform (Mallinckrodt Chemical Works, Analytical Reagent) to yield 1:10, 1:100, and 1:1000 solutions (w/v). The disks, 11 mm. in diameter and prepared from E. & D. No. 615 filter paper, were immersed in the corresponding solutions for a few seconds and the solvent permitted to evaporate in the air. The disks were placed upon agar seeded with the appropriate microorganisms and incubated at 37°. A control was provided by a disk immersed in the solvent and treated as above.

In both methods nutrient agar was used for *Micrococcus pyogenes* and *Escherichia coli* and Sabaroud's agar for *Candida albicans*. The plates were read at twenty-four hours and forty-eight hours. No inhibition of growth by either the N-substituted phosphoroamidothioates or by the two phosphorochloridothioates which inhibited AChase, was observed.

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The results of the experiments performed to determine the *in vitro* biological activity of the compounds used in this investigation toward AChase can be explained on the basis of the electronic theory. It is generally accepted that during a reaction between an alkyl phosphate and cholinesterase the enzyme becomes phosphorylated (12, 23). There is also evidence that the phosphate is split in this reaction (12, 24).

The phosphorylation of the enzyme can be rationalized to proceed in a stepwise manner as represented in Fig. 4.

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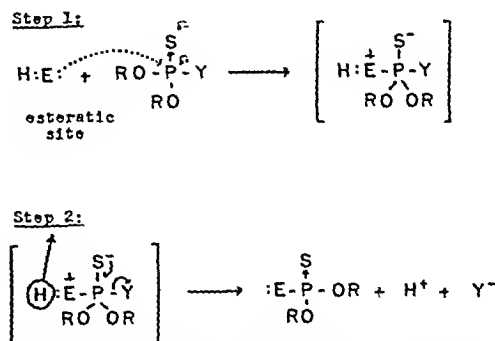


Fig. 4.—Electronic mechanism for the phosphorylation of AChase by an alkyl phosphate type inhibitor. Y—strongly electronegative group or atom.

Since the determined I_{50} values for the two phosphorochloridothioates are very close to each other, it can be concluded that the presence of a methyl group in di-(*p*-tolyl) phosphorochloridothioate does not influence the electrophilic character of the phosphorus atom to any significant degree. Further-

more, the chlorine atom in the P—Cl bond exerts a greater inductive effect than either the phenoxy or the 4-methylphenoxy groups (25), and thus this bond is easily broken and the chlorine expelled as the chloride ion.

The absence of any anticholinesterase activity observed in the thirteen N-substituted phosphoramidothioates synthesized in this investigation suggests the inability of these compounds to phosphorylate the enzyme. An examination of the electronic configuration of a phosphoramidothioate molecule reveals that the phosphorus atom does not possess an electrophilic character due to the proximity of a nitrogen atom with two free electrons (Fig. 5). Therefore, the first step in the phosphorylation reaction (Fig. 1) cannot take place and the inhibition of the enzyme does not occur.

In the experiments designed to determine the activity of the compounds toward microorganisms no inhibition of growth was observed. The negative results of this part of the investigation could be explained on the basis of either the extreme insolubility of these compounds in water or the unfortunate choice of the test organisms.

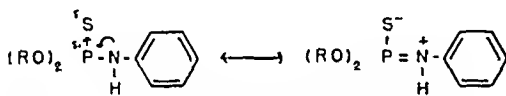


Fig 5 —Resonance forms of an N-substituted phosphoramidothioate

The work of Hood and Lange (21) demonstrated that the sodium salts of the "half esters" of monofluorophosphoric acid inhibited the growth of *Aspergillus niger* and *Aspergillus flavus* but were inactive toward bacteria and yeasts. It is possible, therefore, that *Micrococcus pyogenes* var. *aureus*, *Escherichia coli* and *Candida albicans* which were used in this work to investigate the biological activity of the prepared compounds do not possess enzyme systems affected by diphenyl phosphorochloridothioate, di-(*p*-tolyl) phosphorochloridothioate or by either one of the N-substituted phosphoramidothioates.

In the germination experiments it was found advisable to deposit a given volume of the ethanolic solution of the compound in the center of a filter paper disk, permit the solvent to evaporate, and then use the setup described by Ramaswami, *et al.* (22), but substitute distilled water for the dilute aqueous solution of the compound. This modification was adopted after several preliminary experiments demonstrated that even a 0.5% concentration of the solvent (ethanol) in distilled water partially retarded the germination of both the mono- and the dicotyledonous plants under the experimental conditions reported by Ramaswami.

The germination of the mono- and the dicotyledonous plants used in this investigation was not affected by either the two phosphorochloridothioates or by the two phosphoromethylamidithioates which were chosen at random out of the thirteen N-substituted phosphoramidothioates prepared.

In view of the fact that the dialkyl anilidophosphates investigated by Ramaswami, *et al.*, retarded to varied degrees the growth of the roots and the shoots of a monocotyledonous plant, the negative

results obtained with diphenyl phosphoro-(*m*-toluido)-thioate and diphenyl phosphoro-(*p*-toluido)-thioate can be attributed either to the stereochemical differences between the above compounds as compared to those investigated by Ramaswami or to the extreme insolubility of the two phosphoromethylamidithioates in water and thus the inability to provide a high enough concentration of the inhibitor.

Since neither the two cholinesterase inhibitors; i.e. diphenyl and di(*p*-tolyl) phosphorochloridothioates had any effect on the germination of the mono- or the dicotyledonous plant used in these experiments, it would be safe to assume that the insolubility in water rather than the total absence of a suitable enzyme system was also responsible for the inability of these compounds to influence the germination process. Although hemicellulase and diastase are, probably, the first enzymes which develop during the initial phase of the germination process, a number of other enzymes must be responsible for the development of the shoots and roots (26).

It is highly improbable that these two phosphorochloridothioates are specific cholinesterase inhibitors while a number of closely related organophosphorus compounds is active toward several other enzymes (6), some of which are known to be present in plant cells (26).

It is concluded that the extreme insolubility in water of the phosphoromethylamidithioates and the phosphorochloridothioates is responsible for the failure of these compounds to retard the growth of the roots and shoots of the two particular plant species. Nevertheless, the stereochemical difference between the former compounds and those tested by Ramaswami, *et al.*, should not be underestimated if one keeps in mind the extreme stereospecificity of chemical reactions in biological systems.

REFERENCES

- (1) Schrader, G. *Angew. Chem.*, **62**, 471 (1950).
- (2) McCombie, H., and Saunders, B. C., *Nature*, **157**, 287 (1916).
- (3) Kilby, H. A., *Research*, **2**, 317 (1919).
- (4) Kilby, H. A., *Brit. J. Pharmacol.*, **2**, 234 (1947).
- (5) Adrain, E. D., *et al.*, *ibid.*, **2**, 56 (1917).
- (6) Aldridge, W. N., *Chem. & Ind. (London)*, 473 (1954).
- (7) Augustinsson, K. B., *Acta Chem. Scand.*, **6**, 959 (1952).
- (8) Todrick, A., *et al.*, *Biochem. J.*, **48**, 360 (1951).
- (9) O'Brien, R. D., *J. Biol. Chem.*, **219**, 927 (1956).
- (10) Wilson, I. B., and Meislich, E. K., *J. Am. Chem. Soc.*, **75**, 4628 (1953).
- (11) Wagner-Jauregg, T., *Arzneimittel-Forsch.*, **6**, 194 (1956).
- (12) Wilson, I. B., and Bergmann, F., *J. Biol. Chem.*, **185**, 179 (1950).
- (13) Bruer, R. W., *J. Pharmacol. Exptl Therap.*, **92**, 162 (1918).
- (14) Anienrieth, W., and Hildebrand, O., *Ber.*, **31**, 1094 (1898).
- (15) "Peroxide Bomb Apparatus and Methods, Manual No. 121," Part Instrument Co., Moline, Ill., 1950, p. 27.
- (16) Steyermark, A., "Quantitative Organische Microanalysis," The Blakiston Co., New York, 1951, p. 102.
- (17) Lasso, A., and Rodin, J. S., private communication.
- (18) Fleislin, S., *J. Biol. Chem.*, **180**, 219 (1919).
- (19) Fleischer, J. H., *et al.*, *Anal. Chem.*, **27**, 1080 (1955).
- (20) Ackermann, W. W., and Poller, V. R., *Proc. Soc. Exptl Biol Med.*, **72**, 1 (1949).
- (21) Hood, A., and Lange, W., *J. Am. Chem. Soc.*, **72**, 4956 (1950).
- (22) Ramaswami, D., *et al.*, *Science*, **116**, 58 (1952).
- (23) Burgen, A. S. V., and Hobbinger, F., *Brit. J. Pharmacol.*, **6**, 503 (1951).
- (24) Jansen, E. F., *et al.*, *J. Biol. Chem.*, **179**, 201 (1949).
- (25) Ferguson, L. N., "Electronic Structures of Organic Molecules," Prentice-Hall, Inc., New York, 1952, p. 76.
- (26) Meyer, B. S., and Anderson, D. B., "Plant Physiology," D. Van Nostrand Co., Inc., New York, 1955, p. 289.

Bromide Excretion as Affected by Chloride Administration*

By ANN LANGLEY CZERWINSKI

Blood bromide concentrations in rabbits and dogs were found to be elevated following discontinuance of bromide, not only in animals receiving chloride, but also in controls. Elevation of the blood bromide level in controls was significantly greater than the elevation observed in those receiving chloride. Fluctuations in bromide levels during excretion are taken as evidence that bromide toxicity is related to total bromide extracellular retention rather than simply to blood chloride replacement.

SINCE 1922 bromide intoxication therapy has generally consisted in withholding bromides, forcing fluids, and administering large amounts of sodium chloride(1). In 1930 Wagner and Bunbury (2) reported that as a consequence of chloride administration the blood bromide "usually rises slightly and there is an exacerbation of the toxic symptoms which may prove fatal." They felt that in the presence of debility, dehydration, or a weakened condition, chlorides should be withheld until the bromide level had fallen below a dangerous level. Similar statements may be found in the literature on bromide intoxication (3-7). However, Wuth, Diethelm, and others find no such exacerbation of symptoms upon chloride administration(8-13).

The present work was undertaken to determine whether the blood bromide level falls rapidly upon chloride administration, as reported by Wuth (8) and Diethelm (9), or rather if it is at least temporarily elevated, as claimed by Wagner and Bunbury (2).

EXPERIMENTAL PROCEDURES

Test Animals.—Twelve white rabbits and seven dogs were used. The animals were fed the usual stock diet and allowed free access to water. Blood chloride concentrations and chloride excretion were determined daily until a norm had been established for each animal.

Production of Bromism.—One gram of sodium bromide per Kg. of body weight administered in a 10% solution by stomach tube on three successive days will produce a good degree of acute bromism without endangering the life of the experimental animal. Chronic bromism in rabbits was obtained

by adding enough sodium bromide to the drinking water so that the rabbits received approximately 1 Gm. daily. In dogs 0.3 Gm. of sodium bromide per Kg. of body weight will produce chronic bromism in two weeks.

Chloride Administration.—When definite bromism had been obtained, whether acute or chronic, approximately half of the animals were placed on sodium chloride, the other half being reserved as controls. Whenever possible, upon recovery, the animal was again treated with bromides and the procedure reversed; that is, an animal previously treated with chloride was allowed to recover without chloride. For purposes of comparison, four rabbits used for acute bromism and treated with chloride were subsequently subjected to chronic bromism. Rabbits received 1 Gm. of sodium chloride per Kg. of body weight in a 10% solution daily by stomach tube. Controls received an equal amount of distilled water by stomach tube. Dogs received the same dosage administered in capsules.

Analytical Methods.—Blood chloride or total halides were determined by the modified Vollhard-Harvey titration on a protein-free filtrate (14). Blood bromide was determined by the Greenberg method, in which the bromide of deproteinized whole blood is oxidized to bromate and determined by iodometric titration (15). Urinary chloride and bromide were determined by using these same tests on a diluted portion of urine taken from a well-mixed twenty-four-hour sample. Since urine and blood samples were collected each morning, the urine represents the excretion for the total day preceding the specific blood level set down for that day.

RESULTS

Effect of Chloride on Blood Bromide Level.—The effects of administering sodium chloride to rabbits in a state of bromide intoxication are shown in Table I, to dogs in Table II. The last column gives the increase or decrease in the blood bromide level which occurred between the first and second days following the last dose of bromide.

Seventy-five per cent of the animals on chlorides show a rise in blood bromide level between the first and second day after the last dose of bromide. Of the animals reserved as controls, 70% show a rise in blood bromide. Approximately 85% of the rabbits show an increase in the blood bromide level in-

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The assistance of the following personnel of the Creighton University Animal Hospital is hereby acknowledged: E. L. Freeland, G. B. Green, and Redrick Raglin.

TABLE 1—BLOOD LEVELS IN RABBITS DURING FIRST TWO DAYS OF WITHDRAWAL PERIOD

Rabbit	Blood Levels on 1st Day of Bromide ^a		Blood Levels on 1st Day after Bromide ^a		Blood Levels on 2nd Day after Bromide ^a		Increase or Decrease
	Cl	Br	Cl	Br	Cl	Br	
Acute Bromism Not on Chloride							
A	4 28	0 90	4 15	2 55	3 34	4.00	1 45
B	9 40	3 02	5 40	1 15	5 29	1 71	0 56
E	5 12	0 50	5 06	3 56	4 29	4 46	0 90
F	8 13	0 19	8 04	1 09	4 62	4 42	3 33
H	9 79	0 73	4 44	4 02	3 68	4 22	0 20
I	9 78	0 62	6 54	1 62	6 16	2 46	0 84
F ₁	8 20	0 31	3 38	1 35	4 52	2 32	-2 03
F ₂	1 02	2 02	7 57	0 11	6 61	1 20	1 09
Acute Bromism on Chloride							
A	5 00	2 25	6 60	1 98	11 46	3 85	1 87
E	7 58	1 32	10 73	1 09	8 28	1 40	0 31
I	7 36	0 66	7 31	0 98	8 17	0 81	-0 14
B ₂	5 86	1 20	6 11	0 59	5 60	0 89	0 30
Chronic Bromism Not on Chloride							
C			1 16	1 96	2 46	3 28	1 32
D			5 52	1 17	1 28	5 25	1 08
E			1 87	2 26	3 55	3 26	1 00
B ₁			1 57	3 88	1 60	1 36	-2 52
G ₂	3 71	2 90	1 88	2 11	2 83	3 32	1 18
Chronic Bromism on Chloride							
I			5 02	3 18	2 92	3 44	0 26
F ₂			2 54	2 78	3 79	2 98	0 20
H ₂			5 38	2 50	3 91	3 18	0 68

^a Figures express meq. per 100 ml. of blood.^b Average increase in blood bromide level of rabbits not on chloride, 1 18; average increase in blood bromide level of rabbits on chloride, 0 60.

TABLE 11—BLOOD LEVELS IN DOGS DURING FIRST TWO DAYS OF WITHDRAWAL PERIOD

Dog	Blood Levels on 1st Day of Bromide ^a		Blood Levels on 1st Day after Bromide ^a		Blood Levels on 2nd Day after Bromide ^a		Increase ^b or Decrease
	Cl	Br	Cl	Br	Cl	Br	
Acute Bromism Not on Chloride							
120	5 95	0 27	1 04	1 20	7 00	0 64	-0 56
	3 61	0 70	4 54	2 42	2 19	1 29	-1 13
121	1 10	2 12	6 34	0 81	4 53	2 60	1 79
	5 12	0 72	4 05	2 44	3 20	2 82	0 38
122	5 26	0 71	3 89	2 55	5 83	0 94	-1 61
Acute Bromism on Chloride							
121	6 11	0 20	4 71	2 62	6 16	0 83	-1 79
122	5 23	1 39	4 65	1 43	4 26	2 22	0 79
	3 33	0 51	3 85	2 28	3 08	1 94	-0 34
Chronic Bromism Not on Chloride							
115			3 75	1 63	2 97	2 74	1 11
116			3 05	3 90	2 85	2 52	-1 38
Chronic Bromism on Chloride							
117			3 39	1 81	4 04	1 88	0 07
118			5 58	1 89	4 17	2 10	0 21

^a Figures express meq. per 100 ml. of blood.^b Average increase in blood bromide level of dogs not on chloride, 1 09; average increase in blood bromide level of dogs on chloride, 0 36.

respective of chloride administration. In the dogs, 60% of those on chloride showed an elevation of the blood bromide, while 43% of the controls manifested a higher blood bromide.

The average increase in the blood bromide levels of the individual groups of animals is also significant. For those rabbits on chloride, the average increase was 0 60 meq./100 ml. of blood; while for the con-

trols, the average increase was 1.18 meq./100 ml.—an increase practically twice that of the rabbits on chloride. The dogs on chloride showed an increase in the blood bromide level of 0.26 meq./100 ml. of blood, while the controls showed an increase of 1.09 meq./100 ml.—a difference three times that of the animals on salt.

Only 1 rabbit (B₂) showed an elevated blood bromide on chloride but not when used as a control. Thus, with rabbits, in only one case out of twelve, could the chloride have been said to have caused an elevation of the bromide level. Inspection of Table II shows that in dog No. 121 the blood bromide level was elevated twice during control periods, but was definitely lowered when given sodium chloride. In the case of No. 122, there is only inconclusive evidence, as the blood bromide both rose and fell when chloride was administered.

Effects of Chloride on Amount of Bromide Excreted.—In rabbits chloride administration tends materially to aid bromide excretion (Table III). In acute bromism the rabbits on chloride excreted an average of 1.55 meq. of bromide daily as against 0.42 meq. by the controls. In chronic bromism, those given chloride excreted on the average 1.53 meq. of bromide daily, while controls excreted 0.99 meq. of bromide.

The same ratio prevails with dogs if the intoxication is acute, but not if the bromism is chronic (Table IV).

TABLE III.—BROMIDE EXCRETION IN RABBITS

Rabbit	Time in Days	meq. of Bromide Excreted
Acute Bromism not on Chloride		
A	2	0.04
B	1	3.52
E	2	1.71
F	2	0.51
H	5	0.45
I	2	0.06
F ₂	6	2.36
Average Excretion in 24 hours		0.42
Acute Bromism on Chloride		
A	2	3.75
E	3	4.48
I	3	7.87
B ₂	6	5.56
Average Excretion in 24 Hours		1.55
Chronic Bromism not on Chloride		
C	10	8.12
E	8	9.26
B ₂	10	10.32
Average Excretion in 24 Hours		0.99
Chronic Bromism on Chloride		
I	8	5.21
F ₂	9	13.04
H ₂	8	19.92
Average Excretion in 24 Hours		1.53

TABLE IV.—BROMIDE EXCRETION IN DOGS

Dog	Time in Days	meq. of Bromide Excreted
Acute Bromism Not on Chloride		
122	2	3.84
121	5	1.16
120	7	10.32
Average Excretion in 24 Hours		1.09
Acute Bromism on Chloride		
122	12	20.41
121	2	32.74
Average Excretion in 24 Hours		3.80
Chronic Bromism Not on Chloride		
115	10	5.57
116	10	11.77
Average Excretion in 24 Hours		0.87
Chronic Bromism on Chloride		
117	10	8.85
118	10	9.03
Average Excretion in 24 Hours		0.89

final blood bromide was 0.28 meq./100 ml. of blood, while the average final level in controls was 0.45 meq./100 ml. The average final level in chloride-treated rabbits with chronic intoxication was 0.05 meq./100 ml. while controls showed 0.16 meq./100 ml. of blood. Similar results were seen in the dogs.

Effect of Potassium Nitrate.—One dog was placed on potassium nitrate in an attempt to distinguish between the salt action and the bromide-replacement action of sodium chloride. Although definite conclusions in this case were impossible, it seemed that the potassium nitrate increased the urine volume without increasing the bromide excretion.

Course of Bromide Intoxication in Rabbits and Dogs.—In general, as the blood bromide rises, blood chloride tends to fall. But the course of bromide excretion is not an orderly, gradual decline in the blood bromide. Thus the rise in blood bromide following discontinuance of bromide is followed by several smaller fluctuations in the blood bromide as excretion occurs. Of interest also is the occurrence of low blood bromide levels even during periods when bromide dosage was high, for fluctuations during the production of bromide intoxication are also observed.

DISCUSSION

In the present work blood bromide concentrations were found to be elevated following discontinuance of bromide not only in those animals receiving chloride, but also in the controls. A temporary elevation of blood bromide would seem to be, then, part of the physiological mechanism concerned with bromide excretion, rather than dependent on chloride administration.

During the development of bromide intoxication, the blood absorbs bromide from the gastrointestinal tract and transfers it to the tissue fluids, where it replaces chloride. In the meantime, the kidneys excrete large amounts of chloride, with accompanying small amounts of bromide, in order to maintain osmotic equilibrium of blood and tissue fluids.

Effect of Chloride on Final Blood Bromide Level.—The final blood bromide level in animals receiving chloride was lower than that in controls. In rabbits with acute bromism on sodium chloride, the average

Thus a gradient is established between the blood and tissue fluid which favors deposition of bromide in the tissues. When the system finally reaches equilibrium, as it will with any given dose, the kidneys will excrete approximately the amount of bromide ingested. However, minor fluctuations even during this period will occur, depending principally upon the total halide concentration of the blood. When bromide is discontinued, the equilibrium is upset, and the gradient reversed. The blood now becomes an excretion medium, receiving bromide from the tissues. If the total halide concentration of the blood is not such that bromide excretion is favored, there will result a temporary elevation of blood bromide. Moreover, whenever during the period of withdrawal the blood halide concentration is lowered, subsequent rises in the blood bromide will be observed.

The elevation of the blood bromide in the controls was observed to be significantly greater than the elevation in those receiving chloride. Thus rabbits reserved as controls show a temporary blood bromide rise practically twice that of chloride-treated rabbits, while the degree of blood bromide elevation in untreated dogs is three times that of dogs on salt. Rather than "liberating bromide from the tissues faster than it can be eliminated by the kidney," as postulated by Wagner and Imbry (2), the chloride would seem to hasten urinary excretion of bromide, which is being excreted from the tissues via the blood. Both Bodansky and Modell (16), and Wolf and Radie (17), working separately, have shown that a condition in which the blood is saturated with halide favors bromide excretion. The action of chloride, then, is to lower the blood bromide level by increasing the total halide concentration of the blood and thus facilitate bromide excretion.

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3. Blood bromide concentrations were found to be elevated following discontinuance of bromide not only in those animals receiving chloride but also in the controls. Moreover, the elevation of the blood bromide level in the controls was significantly greater than the elevation observed in those receiving chloride.

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REFERENCES

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Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The number of transformed cells was determined by the number of colonies obtained on the selective medium. The results are the mean of three independent experiments. Error bars represent the standard deviation.

REFERENCES

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Since blood bromide concentrations have been demonstrated in the present work to be elevated following discontinuance of bromide not only in those animals receiving chloride, but also in controls, and since the degree of elevation in the controls is invariably greater than in chloride treated animals, it would seem inadvisable to withhold chloride for fear of elevating the bromide level.

SUMMARY

1 Acute and chronic bromide intoxication were induced in rabbits and dogs with suitable dosage.

2 The level of chloride and bromide in blood and urine of 12 rabbits and 7 dogs showing clinical evidence of bromide intoxication was determined daily for two weeks following discontinuance of bromide to determine whether chloride administration elevated blood bromide levels.

3 Blood bromide concentrations were found to be elevated following discontinuance of bromide not only in those animals receiving chloride but also in the controls. Moreover, the elevation of the blood bromide level in the controls was significantly greater than the elevation observed in those receiving chloride.

4 The blood bromide level was observed to fluctuate considerably during excretion. This is taken as evidence that bromide toxicity is related to total extracellular bromide retention rather than simply to blood chloride replacement. The blood bromide level may be considered to be a function not only of intestinal absorption and kidney excretion, but also of tissue fluid absorption and secretion, which in turn depend upon the total body bromide chloride balance.

REFERENCES

- (1) Harris, T H, and Hauser, A, *J Am Med Assoc*, 95, 94(1930)
- (2) Wagner, C P, and Bunbury, D E, *ibid*, 95, 1725 (1930)
- (3) Perkins, H A, *Arch Intern Med*, 85, 783(1950)
- (4) Strecker, E A, and Ebaugh, F G, "Practical Clinical Psychiatry," ed 5, The Blakiston Company, Philadelphia, Pa., 1940, pp 266-274
- (5) Hanes, F M, and Yates, A, *Southern Med J*, 31, 667(1938)
- (6) Campbell, J D, *ibid*, 42, 967(1949)
- (7) Sensenbaech, W, *J Am Med Assoc*, 125, 769 (1944)
- (8) Wuth, O, *ibid*, 88, 2013(1927)
- (9) Diethelm, O, *J Nervous Mental Disease*, 71, 151, 278(1930)
- (10) Millikan, C H, *J Iowa Med Soc*, 35, 120(1945)
- (11) Wohl, M G, and Robertson, H F, *Penn Med J*, 47, 802(1944)
- (12) Tweed A R, *Am J Psychol*, 2, 650(1948)
- (13) Detweiler, H K, *Can Med Assoc J*, 48, 309(1943)
- (14) Kolmer, J A, and Boerner, F, "Approved Laboratory Technique," ed 4, D Appleton Century Company, New York, 1945, pp 794, 818
- (15) *ibid*, p 914
- (16) Bodansky, U, and Modell, W, *J Pharmacol Exptl Therap*, 73, 51(1941)
- (17) Wolf, R L, and Eadie, G S, *Am J Phys*, 163, 436(1950)
- (18) Sollmann, T, "Manual of Pharmacology," ed 7, W B Saunders, Philadelphia, Pa., 1948, p 809
- (19) Bernoulli, E, *Arch exptl Pathol Pharmacol*, 73, 355(1913)

The Antimicrobial Activity of Perfume Oils*

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One hundred perfume oils were tested for antimicrobial properties against a series of pathogenic and nonpathogenic bacteria and fungi. The *in vitro* filter paper disk method was used exclusively for testing activity. The perfume oils were found to possess remarkable antimicrobial properties with greater activity against fungi than against bacteria. It is suggested that perfume oils be incorporated more widely into common toilet articles applied to the body surfaces as well as medicaments used externally.

HUNDREDS of substances are applied to the skin and hair of the human body for cosmetic and hygienic purposes. Many of these substances are made aromatic to increase their ornamental value and to mask unpleasant odors. Yet the study of the antimicrobial activity of perfume oils has been somewhat neglected. In 1924 Dyche-Teague (1) was apparently the first investigator to demonstrate that alcoholic perfumes exhibited an antibacterial effect. He utilized the phenol coefficient method and as the test organism a mixed culture of bacteria obtained from the nose consisting essentially of *Micrococcus catarrhalis*. In that same year Bryant (2) confirmed the work of Dyche-Teague on the bacterial activity of alcoholic perfumes using as the test

organism a pure culture of *B coli communis*. Recently Lord and Husa (3) found that some perfume oils inhibited mold growth in very low concentrations. These investigators suggested that perfumery materials might be used as preservatives. This report describes the *in vitro* antimicrobial activity of perfume oils against pathogenic and nonpathogenic bacteria and fungi.

MATERIALS AND METHODS

The detection of the antimicrobial activity of 100 perfume oils was made by observing their effects on growing cultures of ten bacteria and ten fungi. The test organisms used were: *Pseudomonas aeruginosa*, *Erwinia caratovora*, *Escherichia coli*, *Serratia marcescens*, *Micrococcus pyogenes* var *aureus*, *Mycobacterium phlei*, *Neisseria flava*, *Bacillus subtilis*, *Sahluella typhosa*, *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Streptomyces tenebriculus*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Cryptococcus rhodabenthami*, *Nigraspora panici*, *Mucor mucedo*, and *Penicillium notatum*. All of the bacteria were cultivated in nutrient agar and nutrient broth (Difco) for twenty-four hours at 37° with the exception of *E. caratovora* which was cultivated in nutrient agar and broth at room temperature for forty-eight hours and *M. phlei* which was cultivated in glucose yeast infusion agar and broth for forty-eight hours at room temperature. All of the fungi were cultivated in Sabouraud dextrose agar and broth (Difco) for forty-eight hours at room temperature with the exception of *C. rhodabenthami*, *N. panici*, and *M. mucedo* which were incubated for one week at room temperature. The mold-like bacterium *S. tenebriculus* was cultivated on nutrient

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agar and broth for forty eight hours at room temperature. Stock cultures of all organisms were subcultured on fresh agar slants every seven days.

The method used for determining the presence or absence of antimicrobial activity was a slight modification of Vincent's (4) qualitative filter paper disk diffusion plate method. In this method small sterile disks of filter paper (12.7 mm diameter) were thoroughly moistened in the oil to be tested and placed on the agar plates (100 mm diameter) which had been previously seeded with 1 cc of the broth culture of the organism. The plates with bacteria were incubated at 37° for twenty-four hours except *E. carotovora* and *M. phlei* which were incubated at room temperature for forty-eight hours. The plates with the fungi were incubated at room temperature for forty eight hours with the exception of *C. rhodopenhani*, *N. panici*, and *M. mucedo* which were incubated for one week at room temperature.

The presence of a definite zone of inhibition of any size surrounding the paper disks indicated antimicrobial activity. The zones of inhibition were measured to the nearest mm by means of a metric ruler and an illuminated Quebec colony counter. All tests were conducted in triplicate with three disks (sometimes two) per dish. Therefore the measurement of each zone of inhibition represents the mean value of at least six recordings. In some instances the oils produced completely clear dishes. When this occurred larger dishes (150 mm diameter) were used in repeating the experiment. In no instance did the oils produce complete clearing of the larger dishes.

All of the zones of inhibition were tested to ascertain whether the activity was microbicidal or microbistatic. This was accomplished by making transfers from the clear zones into broths and incubating for four days or more. Growth in the tubes was indicative of microbistatic activity while no growth indicated microbicidal activity.

As the oils arrived at the laboratory they were immediately placed into sterile bottles and tested for sterility. The test for sterility consisted of streaking each oil on nutrient agar and Sabouraud dextrose agar slants. The nutrient agar slants were incubated at 37° for three to four days while the Sabouraud dextrose agar slants were incubated for one week or more at room temperature. All of the perfume oils were found to be free of microorganisms.

RESULTS AND DISCUSSION

Of the 100 perfume oils tested, all were found to exhibit antibacterial activity on at least two of the ten organisms employed. Table I lists these oils with their antibacterial activity and zones of inhibition. From Table I it may be observed that 75% of the perfume oils tested were found to be bactericidal, the remainder bacteriostatic. It is interesting to note that 60% of the oils tested against *B. subtilis* and *B. brevis* proved to be bacteriostatic in contrast to the remaining organisms where 10 to 25% of the oils exhibited bacteriostatic activity. Why the spore forming organisms *B. subtilis* and *B. brevis* should resist being killed by the perfume oils is not known. The answer may lie in the fact that these organisms were found to produce many spores after twenty four hours of growth. Thus the oil

may not be able to penetrate the spore wall as readily as the vegetative cell wall.

Table II lists one hundred perfume oils and their antifungal activity and zones of inhibition. These are the identical oils tested against bacteria in Table I. From Table II it may be observed that all of the perfume oils exhibited antifungal activity on at least six of the ten organisms tested. From Table II it may be observed that more than 80% of the oils tested against fungi were found to be fungicidal while the remainder were fungistatic. By a comparison of the data from Tables I and II 40% of the oils show some antibacterial activity on the 10 bacteria while 84% of the oils exhibited some antifungal activity on the 10 fungi. This seems to indicate that fungi are more vulnerable to perfume oils than bacteria. Table III lists the most effective perfume oils against each test organism. Thus Almond S exhibits maximum effectiveness against *E. carotovora*, *S. venezuelae*, *C. rhodopenhani*, and *M. mucedo*, Cinnamon against *N. perflava*, *M. phlei*, and *C. krusei*, Citrus Odor No 50 B against *B. brevis*, *S. cerevisiae*, and *A. niger*, and Lemon Bouquet No 58 against *N. panici*, *P. notatum*, and *C. tropicalis*.

There appears to be little doubt that common toilet articles treated with perfume oils (or their constituents) such as soaps, creams, shampoos, lotions, ointments, powders, sprays, and other such preparations which are applied externally for various hygienic purposes, would also kill microbes in the areas applied. It seems rather fitting that those perfume oils which possess marked antimicrobial properties might be incorporated more widely into medicaments used externally to enhance their aesthetic and germicidal properties as well as to mask unpleasant odors.

CONCLUSIONS

- 1 The antimicrobial activity of 100 perfume oils was tested against growing cultures of 10 bacteria and 10 fungi with the *in vitro* filter paper disk method.

- 2 The perfume oils which exhibited the greatest antimicrobial activity were Almond S against *E. carotovora*, *S. venezuelae*, *C. rhodopenhani*, and *M. mucedo*, Cinnamon against *M. phlei*, *N. perflava*, and *C. krusei*, Citrus Odor No 50 B against *B. brevis*, *S. cerevisiae*, and *A. niger*, Lemon Bouquet No 58 against *C. tropicalis*, *P. notatum*, and *N. panici*, Compounded Fruit Odor No 1285 against *E. coli* and *S. marcescens*, Wisteria against *Ps. aeruginosa*, Pine Bouquet Supreme against *M. aureus*, Neutralizer F A against *M. phlei*, Pine Bouquet Swiss Type against *B. subtilis*, Orange Blossom "N" against *S. typhosa*, Allspice against *P. notatum*, Cologne "F" European Type against *C. albicans*, and Lilac Water against *C. krusei*.

- 3 All of the oils were found to possess antimicrobial activity on at least two of the ten

TABLE I—INHIBITORY ACTIVITY OF PERFUME OILS ON BACTERIA

Oils	Average Zone of Inhibition in mm ^a									
	<i>Ps acru- ginosa</i>	<i>B brevis</i>	<i>E carato- rora</i>	<i>E coli</i>	<i>S marce- scens</i>	<i>M aureus</i>	<i>M phlei</i>	<i>N per- flava</i>	<i>B sub- tilis</i>	<i>S ty- phasa</i>
Allspice	0 ^b	12 ^c	13	10	0	7	27	12	11	0
Almond S	0	0	73	0	0	0	16	0	0	0
Apple Blossom No. 200	1	7	20	6	1	3	15	6	10	7
Apple Blossom B S	1	5	23	6	8	2	12	0	6	5
Arabian "N"	1	4	27	3	4	4	7	4	8	5
Ashton Villa No. 6	2	4	11	4	3	5	13	3	8	5
Bay Rum Essence	3	8	12	8	10	7	29	6	8	9
Blue Bell Bouquet	2	7	20	6	3	4	18	4	7	5
Bluestone Bouquet	2	8	24	5	4	9	8	3	3	4
Bouquet No. 21	0	9	38	7	5	13	24	7	17	8
Bouquet No. 22	0	2	10	2	1	6	8	0	4	2
Bouquet 821 Lemon Odor	0	11	12	8	4	10	10	8	15	8
Bouquet B L S	0	7	15	7	1	5	10	6	10	10
Carnation No. 1162	3	4	15	3	6	4	6	3	5	3
Chypre, French Type	1	3	7	2	3	3	5	1	2	3
Chypre 66D	0	2	35	0	0	2	2	0	2	0
Cinnamon	2	0	28	0	0	8	35	23	15	0
Citrus Odor No. 50B	0	20	5	0	0	0	20	0	24	0
Cologne, American	3	10	21	3	4	9	10	3	4	5
Cologne "F," European Type	0	8	43	3	3	7	15	3	3	4
Colonial Bouquet	0	3	10	0	0	0	15	2	6	4
Compounded Fruit Odor, No. 1285	5	13	15	12	21	6	30	8	17	11
Caryopsis No. 602	3	4	12	4	5	0	14	5	5	5
Caryopsis No. 604	3	5	25	5	5	6	8	4	4	5
Crab Apple Blossom	3	5	15	2	3	5	8	2	3	5
Eau de Cologne "S"	0	5	3	3	0	3	15	0	20	2
Eau de Quinine M.O.	0	4	5	5	3	4	7	3	2	4
Elder Buds	2	4	24	4	4	3	30	0	9	6
Evergreen Bouquet	0	2	25	0	0	0	10	0	3	0
Florida Water	2	9	33	6	2	5	8	3	9	4
Fougere No. 966	0	3	8	3	1	3	7	2	5	2
Fragipanni	3	7	15	4	0	6	9	4	8	5
Gardenia No. 1500	2	8	34	6	3	5	20	3	9	5
Gardenia G H.	0	3	15	3	3	3	6	3	4	0
Gardenia J.M.	1	5	13	3	4	3	7	0	7	5
Gardenia "S"	2	6	29	7	0	9	7	7	11	8
Geranium Bouquet	0	4	8	2	2	4	8	2	2	2
Heliotrope	3	5	30	0	4	6	8	4	5	4
Honey Suckle	5	3	20	5	5	4	6	4	6	6
Hyacinth "N"	0	4	14	0	4	0	13	3	5	0
Jasmine No. 11347	2	6	26	5	0	2	8	5	9	9
Jasmine No. 679	0	3	25	2	2	3	5	2	2	2
Jasmine "N"	2	10	27	0	10	8	15	10	12	7
Jasmine Ordinary	2	3	7	3	3	2	8	2	4	4
Jockey Club	1	4	24	3	2	6	10	2	5	4
Lavender "Y"	0	3	24	4	2	8	6	2	11	4
Lavender Bouquet D R	0	5	15	3	2	10	11	2	9	4
Lemon Bouquet No. 58	0	0	12	0	3	0	10	0	20	3
Lemon Bouquet No. 62	0	0	10	5	0	4	26	3	9	4
Lemon L M.	0	6	5	5	0	3	18	2	6	3
Lilae Royal	3	7	20	10	10	5	16	7	10	6
Lilae Water	3	6	17	0	0	0	11	3	7	5
Lilas Blanc	2	5	15	0	1	0	15	3	6	3
Lilas Blanc L S.	3	6	14	8	7	7	30	6	10	7
Lilas Vegetal	2	10	21	10	8	7	25	7	10	8
Lilly of the Valley, Supreme	2	7	15	8	9	8	20	8	10	5
Lotus Blossom	3	7	10	10	8	10	30	7	8	8
Magnolia	2	5	14	8	6	6	6	5	7	7
May Apple Blossom	4	7	22	5	7	4	20	4	9	6
Mille Fleurs	3	8	13	8	8	8	22	5	10	12
Mimosa No. 11548	3	10	17	7	10	3	15	5	11	9
Mint Bouquet No. 122	0	5	12	6	5	6	18	3	6	5
Nareissus	3	9	10	8	9	5	23	6	8	10
Neroli Artificial	0	5	12	0	1	0	20	3	7	3
Neutralizer, F A	4	15	27	10	11	13	35	10	18	12
New Mown Hay, No. 100	4	7	12	8	6	7	20	5	7	8
Orange Blossom "N"	0	4	14	12	5	6	25	5	10	19
Oriental Bouquet No. 225	0	4	15	4	2	5	12	3	6	0
Osheana	0	2	16	1	1	3	16	1	3	2
Palma Bouquet	0	3	10	4	3	0	12	2	4	3
Pine Needle Bouquet No. 400	0	0	3	2	0	0	23	0	0	0

TABLE 1. (continued)

Oils	Average Zone of Inhibition in mm ^a									
	<i>Ps. aerugi- nosa</i>	<i>B. brevis</i>	<i>E. carato- vora</i>	<i>E. coli</i>	<i>S. marce- scens</i>	<i>M. aureus</i>	<i>M. phlei</i>	<i>N. per- flava</i>	<i>B. sub- tilis</i>	<i>S. ty- phosa</i>
Pine Needle Bouquet V H.	0	0	2	0	0	0	15	0	0	0
Pine Bouquet, Supreme	0	3	7	4	2	15	15	2	8	3
Pine Bouquet, Swiss Type	0	4	5	5	0	4	8	2	29	3
Rose No. 81412, Otto Type	0	4	8	3	1	3	12	2	5	3
Rose D.B.	0	5	15	0	7	3	8	6	4	4
Rose Bleue	3	8	17	6	10	3	18	4	9	5
Rose Briar	0	5	17	5	3	3	12	2	5	5
Rose Gladis	2	9	22	3	6	4	33	5	11	1
Rose Odorata	0	8	10	5	6	6	14	5	9	6
Rose Red	4	9	33	4	0	7	17	7	11	7
Roxul Bouquet	0	4	5	6	2	2	6	2	8	6
Rose No 225	0	5	9	3	1	1	14	2	4	3
Rosesol	0	0	4	1	1	0	3	0	2	0
Russian Leather	0	3	14	3	0	3	3	1	4	2
Sandalwood A-3	3	6	10	7	9	10	13	7	9	10
Spring Flower Bouquet	6	10	10	7	0	9	6	6	10	8
Sweet Grass	0	4	43	5	3	5	25	0	5	0
Sweet Pea	2	10	11	9	5	6	10	6	13	7
Trefle	0	5	25	5	0	6	6	2	9	5
Tuberose	5	10	12	9	10	7	35	7	10	8
Vanilla K-600	0	3	8	3	2	3	5	2	2	5
Violet No 23	3	5	17	6	6	6	8	6	8	6
Violet No 257	3	5	13	5	5	6	15	4	5	6
Violet "F"	2	2	10	3	3	5	11	2	3	0
Violet F D	5	8	11	8	5	8	5	7	10	8
Violet de Luxe	1	4	5	3	3	3	7	2	6	3
Violet, Supreme	1	6	10	5	3	4	8	3	9	4
Wisteria	9	8	10	11	12	9	5	8	10	10
Ylang Ylang, Artificial	0	4	3	4	2	0	2	0	3	4

^a Measurement from disk edge to zone edge^b Zone of inhibition absent^c Zones inhibition in italics indicate bacteriostatic activity All other zones in the table indicate bactericidal activity.

TABLE 11.—INHIBITORY ACTIVITY OF PERFUME OILS ON FUNGI

Oils	Average Zone of Inhibition in mm ^a									
	<i>C. albicans</i>	<i>C. tropi- calis</i>	<i>C. krusei</i>	<i>S. tenu- zuelae</i>	<i>S. cere- viscae</i>	<i>A. niger</i>	<i>C. rhodo- benhans</i>	<i>N. panici</i>	<i>M. mu- cudo- tum</i>	<i>P. nota- tum</i>
Allspice	12	6	5	2	12	27	24	20	27	50
Almond S	0 ^b	0	5	40	9	22	90 ^c	45	78	29
Apple Blossom, No. 200	5	7	5	4	11	15	15	18	16	15
Apple Blossom, B.S.	6	7	3	5	7	8	10	10	7	12
Arabian "N"	8	5	5	9	7	15	12	24	11	12
Ashton Villa No. 6	5	10	5	8	5	15	10	25	7	15
Bay Rum Essence	9	7	7	8	11	18	20	20	12	20
Blue Bell Bouquet	7	10	8	10	18	15	14	26	12	20
Bluestone Bouquet	8	10	6	10	12	27	14	38	20	20
Bouquet No. 21	8	8	4	10	10	17	33	22	4	21
Bouquet No. 22	3	5	2	9	2	7	10	6	6	10
Bouquet 821 Lemon Odor	15	6	7	17	12	20	26	22	20	20
Bouquet B.L.S.	10	7	7	9	5	20	20	30	7	20
Carnation No. 1162	5	6	5	6	8	20	14	20	10	15
Chypre, French Type	5	5	5	7	7	11	6	9	7	10
Chypre 66 D	0	1	2	3	3	5	6	4	2	6
Cinnamon	9	7	10	11	25	15	20	38	23	20
Citrus Odor No. 50 B	3	15	5	25	30	35	4	25	4	45
Cologne, American	5	6	8	13	10	18	12	25	20	15
Cologne "F," European Type	20	9	5	14	10	20	9	25	25	13
Colonial Bouquet	7	8	8	12	12	20	8	40	6	20
Compounded Fruit Odor 1285	8	7	7	12	12	8	25	25	3	14
Caryopsis No. 602	6	10	8	11	15	16	10	32	16	20
Caryopsis No. 604	7	10	5	11	10	15	12	20	15	15
Crab Apple Blossom	8	10	5	8	8	21	15	30	10	18
Eau de Cologne "S"	4	3	2	4	5	3	7	25	10	3
Eau de Quinine, M.O.	7	4	3	10	4	20	12	18	15	15
Elder Buds	9	7	4	12	12	16	12	18	20	20
Evergreen Bouquet	0	3	0	10	0	8	7	7	3	15
Florida Water	8	5	5	5	11	18	12	17	14	15
Fougere No. 966	0	3	3	2	3	10	12	20	6	15
Fragipanni	5	5	0	9	13	14	14	17	10	15

TABLE II. (continued)

Oils	Average Zone of Inhibition in mm ^a									
	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>A. niger</i>	<i>C. rhodopenensis</i>	<i>N. panici</i>	<i>M. mucedo</i>	<i>P. notatum</i>
Gardenia No 1500	0	6	5	9	9	15	12	20	9	15
Gardenia, G H	6	7	8	10	15	13	10	20	10	20
Gardenia, J M	5	6	0	8	9	15	14	17	11	18
Gardenia "S"	8	7	5	10	14	10	17	30	15	20
Geranium Bouquet	8	5	3	13	4	15	10	25	13	20
Heliotrope	6	7	7	10	10	14	15	30	10	18
Honey Suckle	7	5	7	9	7	15	10	25	5	18
Hyacinth "N"	5	8	7	10	12	14	11	20	14	20
Jasmine No 11347	6	7	7	10	6	15	12	20	15	15
Jasmine No 679	3	5	2	7	4	12	6	11	5	10
Jasmine "N"	5	8	0	10	17	15	14	22	17	18
Jasmine Ordinary	2	6	3	8	6	20	6	7	9	20
Jockey Club	5	6	5	10	12	16	15	20	18	15
Lavender "Y"	14	7	0	10	8	25	15	26	15	20
Lavender Bouquet D.R	8	3	3	12	10	15	11	25	15	15
Lemon Bouquet No 53	5	10	7	12	10	25	30	50	8	50
Lemon Bouquet No 62	8	3	3	8	7	8	9	8	3	15
Lemon L M	7	6	3	5	4	8	11	20	5	17
Lilac Royal	10	6	6	8	20	13	10	25	6	18
Lilac Water	8	10	10	13	20	20	15	34	22	15
Lilas Blanc	6	10	9	12	20	23	17	35	5	20
Lilas Blanc, L S.	8	10	8	11	7	17	20	25	5	15
Lilas Vegetal	10	10	5	5	14	15	17	30	6	15
Lilly of the Valley, Supreme	5	8	6	4	10	14	21	30	9	15
Lotus Blossom	9	8	4	10	8	12	15	20	6	18
Magnolia	7	6	3	10	8	6	12	8	5	13
May Apple Blossom	5	6	5	8	25	10	10	15	6	15
Mille Fleurs	4	6	4	4	8	9	20	8	16	17
Mimosa No. 11548	6	7	3	9	10	10	9	20	4	13
Mint Bouquet No. 122	7	8	3	3	8	10	17	16	4	15
Narcissus	6	7	5	4	24	12	14	8	5	15
Neroli, Artificial	0	7	8	12	11	23	20	35	7	15
Neutralizer F.A	15	12	8	20	20	22	23	42	15	20
New Mown Hay, No. 100	5	9	5	8	10	10	11	17	5	15
Orange Blossom "N"	0	6	4	7	5	15	20	35	11	30
Oriental Bouquet No 225	5	10	5	9	10	35	14	40	6	20
Osheana	3	3	2	5	2	5	5	7	3	10
Palma Bouquet	3	8	5	10	15	20	13	32	10	25
Pine Needle Bouquet No 400	0	0	0	16	15	5	5	2	15	10
Pine Needle Bouquet, V H	0	0	0	3	3	3	2	0	5	2
Pine Bouquet, Supreme	3	4	2	14	3	15	10	10	15	20
Pine Bouquet, Swiss Type	0	0	0	8	7	3	10	10	7	10
Rose No 81412, Otto Type	3	8	3	8	5	12	9	22	3	25
Rose D B	6	6	0	8	10	15	14	30	15	18
Rose Bleue	6	5	4	5	6	10	12	15	5	20
Rose Briar	4	7	4	13	10	20	14	33	15	20
Rose Gladis	9	6	4	3	10	12	20	7	8	25
Rose Odorata	7	10	5	8	8	10	14	25	12	20
Rose Red	10	10	7	9	10	14	18	35	10	20
Royal Bouquet	5	4	2	9	3	12	7	10	4	20
Rose No 225	2	3	3	4	8	6	5	5	3	15
Rosesol	3	0	1	4	3	5	2	0	0	5
Russian Leather	3	4	2	4	8	5	7	5	3	9
Sandalwood A-3	2	4	3	4	10	7	3	10	8	10
Spring Flower Bouquet	7	7	5	10	12	11	14	20	9	7
Sweet Grass	3	8	6	11	10	24	10	37	21	20
Sweet Pea	8	6	5	10	22	14	15	23	13	15
Trefle L H	7	5	3	8	7	10	11	20	9	15
Tuberose	6	7	6	10	11	15	14	22	10	17
Vanilla K-600	4	5	4	8	10	10	8	10	10	11
Violet No 23	5	3	3	9	12	8	8	10	6	10
Violet No 257	3	3	3	9	10	5	8	12	7	10
Violet "F"	3	5	4	8	10	10	8	10	10	11
Violet F D	8	8	5	8	12	12	15	19	5	15
Violet de Luxe	2	2	2	5	5	2	5	4	3	5
Violet, Supreme	3	2	3	8	4	5	7	10	6	10
Wisteria	8	6	6	10	12	17	15	25	3	16
Ylang Ylang, Artificial	4	3	2	6	2	6	2	4	2	10

^a Measurement from disk edge to zone edge^b Zone of inhibition absent^c Zones of inhibition in italics indicate fungistatic activity. All other zones in the table indicate fungicidal activity

TABLE 111—THE EFFECTIVENESS OF PERFUME OILS ON INDIVIDUAL ORGANISMS^a

<i>Ps aeruginosa</i>	(2) Bouquet 821 Lemon Odor and Neutralizer F.A.
(1) Wisteria ^b	(3) Lavender "Y"
(2) Spring Flower Bouquet	<i>C. tropicalis</i>
(3) Compounded Fruit Odor No 1285, Honey Suckle, Tuberosc, and Violet F D	(1) Citrus Odor No. 50 B
<i>B brevis</i>	(2) Neutralizer F A
(1) Citrus Odor No 50 B	(3) Ashton Villa No. 6, Blue Bell Bouquet, Blue-stone Bouquet, Caryopsis No 604, Crab Apple Blossom, Lemon Bouquet No 58
(2) Neutralizer F A	Lilac Water, Lilas Blanc L S, Lilas Vegetal, Oriental Bouquet No. 225, Rose Odorata, and Rose Red
(3) Compounded Fruit Odor No 1285	<i>C. krusei</i>
<i>E caratovora</i>	(1) Cinnamon and Lilac Water
(1) Almond S	(2) Lilas Blanc
(2) Cologne "F" European Type and Sweet Grass	(3) Blue Bell Bouquet, Cologne American, Colonial Bouquet, Caryopsis No 602, Gardenia G H, Lilas Blanc L S, Neroli Artificial, and Neutralizer F A.
(3) Bouquet No 21	<i>S. venezuelae</i>
<i>E coli</i>	(1) Almond S
(1) Compounded Fruit Odor No 1285 and Orange Blossom "N"	(2) Citrus Odor No 50 B
(2) Wisteria	(3) Neutralizer F.A.
(3) Lilac Royal, Lilas Vegetal, Lotus Blossom, and Neutralizer, F A	<i>S. cerevisiae</i>
<i>S marcescens</i>	(1) Citrus Odor No 50 B
(1) Compounded Fruit Odor No 1285	(2) Cinnamon and May Apple Blossom
(2) Wisteria	(3) Narcissus
(3) Neutralizer F A	<i>A. niger</i>
<i>M aureus</i>	(1) Citrus Odor No 50 B and Oriental Bouquet No 225
(1) Pine Bouquet Supreme	(2) Allspice and Bluestone Bouquet
(2) Bouquet No 21 and Neutralizer F A	(3) Lavender "Y" and Lemon Bouquet No 58
(3) Bouquet 821 Lemon Odor, Lavender Bouquet D R, Lotus Blossom, and Sandalwood A-3	<i>C. rhodopenhans</i>
<i>M phlei</i>	(1) Almond S
(1) Cinnamon, Neutralizer F A, and Tuberosc	(2) Bouquet No 21
(2) Rose Gladis	(3) Lemon Bouquet No 58
(3) Compounded Fruit Odor No 1285, Elder Bud, Lilas Blanc L S, and Lotus Blossom	<i>N. panici</i>
<i>N. perflava</i>	(1) Lemon Bouquet No 58
(1) Cinnamon	(2) Almond S
(2) Neutralizer F A and Jasmine N	(3) Neutralizer F A.
(3) Bouquet 821 Lemon Odor, Compounded Fruit Odor No 1285, and Lilly of the Valley Supreme	<i>M. mucido</i>
<i>B subtilis</i>	(1) Almond S
(1) Pure Bouquet Swiss Type	(2) Allspice
(2) Citrus Odor No 50 B	(3) Cologne "F" European Type
(3) Eau de Cologne "S" and Lemon Bouquet No 58	<i>P. notatum</i>
<i>S. typhosa</i>	(1) Allspice, and Lemon Bouquet No 58
(1) Orange Blossom "N"	(2) Citrus Odor No 50 B
(2) Mille Fleurs and Neutralizer F A	(3) Orange Blossom "N"
(3) Compounded Fruit Odor No. 1285	
<i>C. albicans</i>	
(1) Cologne "F" European Type	

^a Data taken from Tables I and II^b Oils arranged in the order of decreasing activity.

bacteria used and at least six of the ten fungi employed

4. The most resistant bacterium was *Pseudomonas aeruginosa* while the most vulnerable was *Erwinia caratovora*. *Candida krusei* was found to be the most resistant fungus while the most susceptible was *Nigrospora panici*.

5. More than 70% of the perfume oils produced microbicidal activity.

6. The antifungal activity of perfume oils

does not parallel its antibacterial properties.

7. Fungi are almost twice as vulnerable to perfume oils as bacteria.

REFERENCES

- (1) Dyche Teague, F. C., *Perfumery Essent Oil Record*, 15, 6(1924)
- (2) Bryant, J. J., *ibid*, 15, 426(1924).
- (3) Lord, J. J., and Husa, W. J., *THIS JOURNAL*, 43, 438 (1954)
- (4) Vincent, J. G., and Vincent, H. W., *Proc Soc. Exptl Biol Med*, 55, 162(1944)

A Comparison of Human Serum Levels of Acetylsalicylic Acid, Salicylamide, and N-Acetyl-*p*-Aminophenol Following Oral Administration*

By JOHN H. WEIKEL, Jr.

Comparative blood levels of acetylsalicylic acid, salicylamide, and N-acetyl-*p*-aminophenol were obtained from the same human subjects following the oral administration of 1.0 gram of each drug. A peak level of 7 mg. per cent was demonstrated for acetylsalicylic acid three hours after ingestion of the drug. No detectable free salicylamide was found in serum, but conjugated salicylamide reached a peak concentration of 2 mg. per cent at one hour. N-Acetyl-*p*-aminophenol showed a peak concentration of about 1 mg. per cent at one hour which was well maintained for the five-hour observation period. These data suggest that, since N-acetyl-*p*-aminophenol exists in its free form, lower blood levels are required for therapeutic effectiveness than with acetylsalicylic acid and salicylamide.

BOTH CLINICAL (1-3) and experimental (3, 4) studies have demonstrated that acetylsalicylic acid, salicylamide, and N-acetyl-*p*-aminophenol exert their antipyretic-analgetic effectiveness with similar oral dosages. A precise and objective measure of this pharmacologic effect is difficult to obtain. However, a comparison of the data on antipyretic or analgetic efficacy with the blood levels of the agents should permit their more definitive characterization. A number of reports on the metabolism of these three drugs appeared in the literature but none of them gives a direct quantitative comparison of the blood concentrations of these antipyretic agents. The various studies on each of the several agents have employed different doses, species, time intervals for blood samples, and experimental procedures.

The following study was instituted to obtain a direct comparison of the blood levels following oral administration of equal quantities of acetylsalicylic acid, salicylamide, and N-acetyl-*p*-aminophenol which might permit a correlation between blood level and pharmacological efficacy. Blood concentrations were determined in human subjects each of whom had ingested each of the drugs on three different days.

EXPERIMENTAL

Six normal, healthy men weighing 153.5 to 195 pounds were used as subjects. Each individual received three 1/2-Gm. capsules of either acetylsalicylic acid (ASA), salicylamide (SAM), or N-acetyl-

p-aminophenol (NAPAP) two hours following a light standardized hospital breakfast. Blood samples were obtained at one-half, one, two, three, and five hours after ingestion of the drug. The serum (2-3 ml.) was immediately separated and then stored under aseptic conditions for subsequent assay. The experiment was repeated at three- or four-day intervals until all subjects had received each of the three drugs.

Although conflicting evidence has been presented as to the presence of salicylate in the acetylated form in the serum following oral administration (5, 6), it is quite evident that any such nonhydrolyzed acid makes only a minor contribution to the total salicylate concentration. Therefore, the serum levels of acetylsalicylic acid were determined and calculated as salicylic acid by means of the ferric ion-salicylate color reaction following an ethylene dichloride extraction (6, 7). Early reports showed that the serum levels of salicylamide, which is not converted to salicylate, were low and not prolonged (3, 8, 9) but Crampton and Voss (10) contended that this drug was present in the serum of the rabbit as a conjugate and that this conjugate was detectable in the serum longer than was free salicylamide. These data indicated that total salicylamide concentrations might be more significant and the serum levels were determined by the method of Crampton and Voss (10). The blood of the subjects which received NAPAP was assayed for free N-acetyl-*p*-aminophenol by hydrolysis of the N-acetyl group followed by diazotization and coupling with α -naphthol (11).

Acetylsalicylic Acid.—Only barely detectable salicylate levels were present in the serum one-half hour following the ingestion of acetylsalicylic acid. The concentration of salicylate in the serum then increased to a peak of about 7 mg. per cent in from two to three hours. The peak average concentration of the six serums was at three hours, but two of the six subjects showed their highest levels at two hours. A slow decline in serum salicylate concen-

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tration was shown by the fact that at five hours the levels were approximately equal to the two-hour concentration. The complete results of the blood serum analyses are given in Table I.

efficacy. The answer probably lies in the relationship between measured serum level and effective serum level.
It can be assumed that following administration

TABLE I —BLOOD SERUM CONCENTRATIONS OF ACETYLSALICYLIC ACID, SALICYLAMIDE, AND N-ACETYL-*p*-AMINOPHENOL IN HUMAN SUBJECTS AFTER A 1-GM. ORAL DOSE OF EACH DRUG^a

Subject	1/2 Hour				1 Hour				2 Hours				3 Hours				5 Hours			
	ASA	SAM	NAPAP		ASA	SAM	NAPAP		ASA	SAM	NAPAP		ASA	SAM	NAPAP		ASA	SAM	NAPAP	
Jo	1.3	0	0.1		3.2	1.1			3.8	0	0.6		4.2	1.6	0.6		6.0	1.4	0.6	
Me	3.0	2.4	0.6		6.5	3.2	0.4		7.1	3.2			7.4	0	1.0		4.1	0	0.8	
Ak	1.5	2.1	0.4		1.2	3.2	0.5		5.0	1.8	1.0		10.2	2.2	1.2		6.0	1.5	0.8	
Wh	3.0		0.6		6.6	1.2	0.5		6.9	1.6	1.0		6.3		0.8		5.0	0	0.6	
Hu	1.2	0	1.2		5.7	0.3	1.4		7.1	1.8	1.1		6.7	1.5	0.8		5.3	0.9	0.6	
Je	2.6	0	1.5		2.0	2.0	1.6		4.2	2.0	0.9		6.8	0.2	1.0		6.5	0.4	0.5	
Average	2.1	1.0	0.7		4.7	1.9	0.9		5.7	1.7	0.9		6.9	1.1	0.9		5.5	0.7	0.7	
S.E.	0.4	0.6	0.7		0.8	0.5	0.8		0.6	0.1	0.3		0.6	0.1	0.5		0.4	0.3	0.3	

^a In mg. per 100 ml. of serum

Salicylamide—The serum levels of salicylamide were much lower than those observed with acetyl-salicylic acid. The serum of two subjects was examined for free salicylamide, but no free drug was detected. It can thus be assumed that following 1.0 Gm. of SAM there was less than 0.3 mg. of free salicylamide in each 100 ml. of serum at any of the time intervals examined in this study. When the serum was subjected to acid hydrolysis to free conjugated SAM, low but measurable levels of salicylamide were found. The total salicylamide concentration rose to an average peak of 1.9 mg. % at one hour. The concentration of salicylamide then declined to barely detectable levels of 0.7 mg. % at five hours. It should be noted that the sensitivity of this method is such that 0.2–0.5 mg. % is the limit of detection and that its accuracy below 1.0 mg. % is poor.

N-Acetyl-*p*-aminophenol.—The serum concentration of NAPAP following oral administration was different from that found with either of the other agents. A peak concentration of about 1.0 mg. % was reached even more rapidly than with salicylamide, and in contrast to salicylamide, this peak represents unconjugated drug. The blood level remained rather constant at this peak from the first to third hour and then declined slowly. Although the concentrations determined at one-half hour and one hour showed considerable variation, the later values were quite consistent among the six subjects.

DISCUSSION

Examination of the data obtained in this study shows that the serum levels obtained by equal doses of acetylsalicylic acid, salicylamide, and N-acetyl-*p*-aminophenol, which are also approximately equal in relationship to the therapeutic dose, are at great variance. Acetylsalicylic acid gives the highest serum concentration of total measured drug but requires the longest period of time to reach the peak level. Salicylamide reaches a considerably lower peak earlier, while N-acetyl-*p*-aminophenol reaches its highest concentration most quickly but never attains the level of the other two drugs. The question arises concerning the apparent discrepancy between serum concentration and therapeutic

of a drug a dynamic equilibrium of the drug between the blood and the tissues and between the tissues and the effector sites is established. If the blood and tissue levels of the drug are equal, a measure of the blood concentration of that drug will give an estimate of the amount available to exert its effect. If only a portion of the drug in the serum is available to combine with the tissues, the blood level is a less reliable measure of an effective concentration.

In the case of NAPAP the measured concentration in the serum represents only free NAPAP and should be an index to the therapeutically active concentration. This supposition is strengthened by the observation of Brodie and Axelrod (12) that the concentration of NAPAP in serum water equaled that in brain water. Additional evidence of the importance of the ratio of free to bound or conjugated drug is furnished by the data by Boxill, Nash, and Wheeler (4) on antipyretic potency and duration of activity.

The parallel between serum levels and pharmacologic activity is less certain in the case of salicylamide. The values determined here represent total salicylamide and this total is, within the limits of detection, all as a conjugated form. Although this conjugated salicylamide may be in equilibrium with an active drug, it is questionable that it is active *per se*. Seeberg, *et al.* (9), showed that the brain and serum salicylamide concentrations were nearly equal up to 1 hour, but that the brain concentrations then declined even more rapidly than did the serum concentrations.

Smith, *et al.* (6), showed that with acetylsalicylic acid the concentration of salicylate in the brain was less than half that of the serum. Seeberg, *et al.* (9), obtained even greater serum to brain ratios ranging from 17:1 at one-half hour to 8:1 at five hours. One possible explanation of this phenomenon is the high percentage of serum protein bound salicylate (6).

It would seem from the data presented here and that from other literature that serum levels of N-acetyl-*p*-aminophenol represent a good measure of tissue levels, and hence are indicative of therapeutically effective drug. The serum levels of acetylsalicylic acid and salicylamide may be a function of the therapeutic levels but cannot be com-

pared directly with those of N-acetyl-*p*-aminophenol.

SUMMARY

1 Human blood levels of acetylsalicylic acid, salicylamide and N-acetyl-*p*-aminophenol have been compared in the same subjects following an oral dose of 1 gram of each drug.

2 Acetylsalicylic acid exhibited the highest serum concentration (7 mg.%) which was reached most slowly and maintained for the longest period of time

3 No free salicylamide could be detected in the blood serum. Total or conjugated salicylamide was present in the blood serum at a peak concentration of about 2 mg %.

4. The peak concentration of N-acetyl-*p*-aminophenol of about 1 mg.% was reached most quickly.

5. The serum levels of N-acetyl-*p*-amino-

phenol which are reported here represent free drug while the levels of acetylsalicylic acid and salicylamide do not. The implications of this difference are discussed in terms of blood level-pharmacologic effect relationships.

REFERENCES

- (1) Batterman, R. C., and Grossman, A. J., *Fed Proc* 14, 316(1955)
- (2) Wallenstein, S. L., and Houde, R. W., *ibid*, 13, 414(1954)
- (3) Litter, M., Moreno, A. R., and Dann, L., *J Pharmacol Exptl Therap*, 101, 119(1951)
- (4) Boxill, G. C., Nash, C. B., and Wheeler, A. G., *THIS JOURNAL*, 47, 479(1958)
- (5) Smith, M. J. H., *J Pharm and Pharmacol* 5, 81(1953)
- (6) Smith, P. K., Gleason, H. L., Stoll, E. G., and Ogorzalik, S., *J Pharmacol Exptl Therap*, 87, 237(1947)
- (7) Brodie, B. B., Udenfriend, A., and Coburn, A. F., *ibid*, 80, 114(1944)
- (8) Mandel, H. G., Rodwell, V. W., and Smith, P. K., *ibid*, 106, 433(1952)
- (9) Seeberg, V. P., Hansen, D., and Whitney, B., *ibid*, 101, 275(1951)
- (10) Crampton, J. M., and Voss, E., *THIS JOURNAL* 43, 470(1954)
- (11) Brodie, B. B., and Axelrod, J., *J Pharmacol Exptl Therap*, 94, 22(1948)
- (12) Brodie, B. B., and Axelrod, J., *ibid*, 97, 58(1948)

Comparative Pharmacological and Toxicological Evaluation of N-Acetyl-*p*-Aminophenol, Salicylamide, and Acetylsalicylic Acid*

By GALE C. BOXILL, CLINTON B. NASH, and ALLAN G. WHEELER

Acute toxicity studies on N-acetyl-*p*-aminophenol (NAPAP), salicylamide (SAM), and acetylsalicylic acid (ASA) revealed that NAPAP was least toxic (LD₅₀) in rats and guinea pigs while SAM was least toxic in mice. As antipyretic agents NAPAP and ASA were approximately equivalent in potency and both were more effective than SAM. A consideration of the ratio of the minimum neurological deficit dose₅₀ (TD₅₀) and the minimum effective antipyretic dose indicated that NAPAP and ASA had a wider safety margin than SAM. ASA produced tachycardia, pyrexia, polypnea, and an increase in tidal volume. NAPAP and SAM produced little change in blood pH, tidal volume, respiratory rate, and heart rate. Myocardial depression was seen with ASA and SAM but not with NAPAP.

THE NUMBER of reports which have recently appeared in the literature dealing with accumulation (1), sensitivity (2), and toxicity (3-5) of the salicylates initiated a search for drugs which could replace them as antipyretics. Among those drugs considered as possible substitutes were N-acetyl-*p*-aminophenol and salicylamide. Although the analgesic or antipy-

retic activities of these compounds are well documented (6-10), other pharmacological and toxicological actions are less well known.

It was observed during the preliminary studies in this laboratory that the toxicological manifestations of N-acetyl-*p*-aminophenol differed from those reported for salicylamide and aspirin. Because of these differences in toxicity and the lack of adequate pharmacological and toxicological information, a comparative study of N-acetyl-*p*-aminophenol (NAPAP, Tempra)¹, sali-

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¹ Mead Johnson and Company trade mark for N acetyl *p*-aminophenol

cylamide (SAM), and acetylsalicylic acid (ASA) was undertaken

EXPERIMENTAL

Acute Toxicity.—The three compounds used for these evaluations were each administered to mice, rats, and guinea pigs in a 10% gum acacia suspension. The volumes of the suspensions administered ranged from 10–30 ml/Kg for mice, 10–20 ml/Kg for rats, and 10 ml/Kg for guinea pigs. Fasted animals were deprived of food from fifteen to seven teen hours prior to drug administration while the nonfasted groups had access to food until the time of the experiment. All animals had access to water *ad libitum* except during the six hour observation period that immediately followed intubation. Animals were housed in rooms that were maintained at 76–78° F and had access to food and water throughout the remaining 162 hours of observation.

Ten male albino mice (17–30 Gm, Hamilton Laboratory animals H L A strain), ten male albino rats (95–180 Gm, H L A strain) and ten guinea pigs (180–310 Gm) were employed at each dose level. The LD₅₀ values were determined in a manner advocated by Lehman, *et al* (11), and computed according to the method of Litchfield and Wilcoxon (12).

The toxic effects from these compounds were manifested in some type of neurological abnormality and were expressed as the minimal neurological deficit dose (TD₀₁). The end point for the TD₀₁ was determined by the method of Swinyard, Brown, and Goodman (13).

Antipyretic Activity.—The activity of NAPAP, ASA, and SAM was compared in male albino rats by a modification of the method of Smith and Ham bourger (14). One hundred and eighty adult male rats (200–250 Gm, H L A strain) which had been fasted for approximately sixteen hours were rendered febrile by the subcutaneous injection of a 15% aqueous suspension of brewer's yeast.² Animals with a normal resting temperature of less than 98.5° F or more than 100° F were excluded from the experiment. Each animal received 10 ml/100 Gm of body weight of yeast. Animals which did not show more than 1° F increase in rectal temperature five hours after the yeast administration, designated as 0 hour, were eliminated from the experiment. All drugs were administered perorally five hours after the yeast injection as a suspension in 10% gum acacia in a volume of 10 ml/Kg. The doses tested for each drug were 100 mg/Kg, 125 mg/Kg, and 150 mg/Kg. Rectal temperatures were taken by a Tele thermometer 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 hours after the animals had received the antipyretic drug.

Anticonvulsant Activity.—The anticonvulsant activity of the three compounds was assayed by two of the battery of tests reported by Swinyard and associates (13) maximal electroshock seizure pattern (MES), metrazol seizure threshold test (Met).

The test compounds were suspended in 10% gum acacia and administered perorally in a volume of 10 ml/Kg to nonfasted male albino mice (H L A strain) that weighed 18–25 Gm.

² Appreciation is expressed to Dr. R. D. Seeley, Anheuser-Busch, Inc., St. Louis, Mo., for furnishing the yeast used in this study.

Each dose level was studied on 25 animals of which 20 received the drug and 5, the controls, received the vehicle only. The convulsive seizures were produced by 50 mA of current being applied for 0.2 second's duration through Spiegel corneal electrodes. Five different drug animals were shocked at 0.5, 1.0, 2.0, and 4.0 hours.

The number of animals and the method of administration of the test drugs were the same as that described above for the MES test. Five different animals were challenged with 85 mg/Kg (SC) of metrazol 0.5, 1.0, 2.0, and 4.0 hours after receiving the test drug. The animals were observed for clonic convulsions for one hour after being challenged with metrazol. If a clonic convulsion which lasted for five seconds occurred, the drug was considered to have offered no protection to that animal.

Prolongation of Sleeping Time.—A modification of the method of Reinhard (15) was used to evaluate mild central nervous system activity of NAPAP, SAM and ASA. Doses of the compound to be tested were administered orally as a suspension in 10% gum acacia in a volume of 10 ml/Kg to groups of male albino mice (17–24 Gm, H L A strain) which had been fasted for approximately sixteen hours. Thirty minutes later the animals were injected, intraperitoneally, with 65 mg/Kg of pentobarbital sodium. Each dose level consisted of 10 mice.

The average sleeping time of each group of the test animals was compared to the average sleeping time of the control animals and tested for significance by the student's "t" test. The smallest dose which differed significantly ($P = 0.05$) from the controls was determined for each drug.

Cardiovascular and Respiratory Studies.—Cardiovascular and respiratory studies were made on mongrel dogs of either sex anesthetized with barbitol sodium, 275 mg/Kg, I.V. Blood pressure was measured with a mercury manometer and respiration recorded by the Anderson spirometer (16). In order to emphasize the differences in action between these three compounds, a fixed dose of 250 mg/Kg suspended in 0.5% Methocel 400 was given by intragastric injection following laparotomy. Blood samples were taken under oil just prior to and at hourly intervals following the drug injection and blood pH was measured with a Beckman Model H-2 meter. Young cats furnished the hearts for the Langendorff isolated heart studies employing the Anderson Craver technique (17) and perfusion fluid (18) was used as the drug solvent in these experiments.

RESULTS

Acute Toxicity.—Species variation to drug sensitivity was illustrated by our findings. NAPAP elicited a significantly lower mortality level in rats and guinea pigs while SAM produced a lower mortality in mice. The LD₅₀ did not differ significantly between NAPAP and ASA in nonfasted mice or between ASA and SAM in rats. The LD₅₀ values were the lowest for NAPAP in fasted mice and for ASA in guinea pigs.

The TD₀₁'s were significantly lower for SAM than

for ASA in rats and mice or NAPAP in rats and fasted mice. Values did not differ significantly between SAM and NAPAP in nonfasted mice or

between ASA and NAPAP in either species. The results of the toxicity studies are summarized in Tables I and II.

TABLE I.—COMPARISON OF THE LD₅₀ VALUES OF N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYL-SALICYLIC ACID

Drug	Species	Nutritional Status F ^a NF ^b	No of Animals Used ^c	Mean Body Weight, Gm	LD ₅₀ in mg /Kg. with 95% confidence limits—			
					24 Hr	48 Hr	96 Hr	168 Hr
NAPAP	Mouse	F	60	20.6	570 (430–756)	525 (392–703)	525 (392–703)	467 (348–626)
	Mouse	NF	90	22.3	1020 (833–1248)	980 (839–1144)	850 (720–1002)	850 (720–1002)
	Rat	F	40	122.5	4450 (3973–4984)	4400 (3928–4928)	4400 (3928–4928)	3700 (3189–4292)
	Guinea pig	F	60	254.3	3500 (3153–3885)	3000 (2600–3450)	2750	2620
SAM	Mouse	F	40	18.7	1210 (1077–1355)	1200 (1090–1321)	1200 (1090–1321)	1200 (1090–1321)
	Mouse	NF	60	19.4	1590 (1355–1867)	1509 (1355–1867)	1590 (1355–1867)	1500 (1340–1679)
	Rat	F	50	150.0	1100 (921–1310)	1100 (921–1310)	1020 (842–1234)	980 (817–1176)
	Guinea pig	F	100	235.0	1800 (1556–2082)	1750 (1511–2025)	1730 (1516–1974)	1730 (1516–1974)
ASA	Mouse	F	60	20.9	960 (872–997)	960 (872–997)	960 (872–997)	960 (872–997)
	Mouse	NF	50	20.8	1000 (909–1110)	1000 (909–1110)	1000 (909–1110)	1000 (909–1110)
	Rat	F	40	121.0	1600 (1194–2144)	1600 (1194–2144)	1430 (1065–1921)	1430 (1065–1921)
	Guinea pig	F	60	238.7	1095 (972–1238)	1075 (948–1219)	1075 (948–1219)	1075 (948–1219)

^a Fasted animals were deprived of food for fifteen to seventeen hours prior to and for six hours following intubation
^b Nonfasted animals were deprived of food for only six hours immediately following intubation
^c Ten animals were the minimum number utilized on each dose level

TABLE II —COMPARISON OF THE TD₅₀ VALUES OF N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYL-SALICYLIC ACID

Drug	Species	Nutritional Status F ^a NF ^b	No of Animals Used ^c	Mean Body Weight, Gm	TD ₅₀ mg /Kg with Confidence Limits, 24 Hr
NAPAP	Mouse	F	40	20.3	144 (121–171)
	Mouse	NF	30	21.8	185 (151–226)
	Rat	F	30	132.3	268 (213–338)
	Rat	F	40	214.0	342 (282–400)
SAM	Mouse	F	30	20.7	87 (59.6–126.7)
	Mouse	NF	30	20.8	142 (107.7–187)
	Rat	F	30	139.8	71 (59–84.5)
	Rat	F	30	208.8	82 (61–110)
ASA	Mouse	F	30	18.6	158 (129–194)
	Mouse	NF	30	22.1	205 (175–240)
	Rat	F	40	106.1	220 (149–323)
	Rat	F	30	213.8	312 (236–412)

^a Fasted animals were deprived of food for fifteen to seventeen hours prior to and for six hours following intubation
^b Nonfasted animals were deprived of food for only six hours immediately following intubation
^c Ten animals were the minimum number utilized on each dose level

In all species studied the signs of toxicity noted following the administration of NAPAP were hypoactivity and depression accompanied by a decreased respiratory rate. Slight tremors were noted in guinea pigs during a depressed state that occurred prior to a loss of righting reflex. Rats were slightly hyperirritable to audible stimuli during the absence of the righting reflex which occurred at only lethal dose levels. Some animals recovered completely from the CNS depression. Death was due to respiratory failure.

The initial toxic effects from SAM on the three species studied were ataxia and depression which progressed to a state of hypnosis in accordance with a dosage increase. Labored respiration and cyanosis ensued prior to death which was apparently the result of respiratory failure. In mice, either a prolonged period of depression or a period of intermittent episodes of hyperactivity, coarse tremors and a running type of convulsion occurred following recovery from hypnosis. Occasionally the SAM ani-

mals displayed bizarre motions characterized by rapid movements of the head in a vertical or horizontal plane and a rapid circular locomotion. No recovery from these later effects was noted during a three weeks' observation period. Those animals which did not display the bizarre activity and survived exhibited a complete recovery from drug effects.

An initial depression accompanied by a decrease in respiratory rate and ataxia were produced with ASA. Mice and guinea pigs were prone to exhibit hyperirritability to audible and tactile stimulation. These signs of intoxication were followed by tremors, Straub's reaction (mice and rats) and clonic convulsions. Salivation was especially evident in mice. Deaths were the result of respiratory failure following repeated convulsive seizures. No after effects from the drug were noted in surviving animals.

Antipyretic Activity.—The results of the antipyretic action of 100 mg./Kg., 125 mg./Kg., and 150 mg./Kg., perorally, of NAPAP, SAM, and ASA are presented in Table III.

TABLE III —ANTIPYRETIC ACTIVITY OF N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYSALICYLIC ACID

Drug ^a	No. Animals	Dose mg./Kg.	Average Temperature After Drug (Hr.), °F.							
			Control -5	0	1/2	1	2	3	4	5
NAPAP	19	150	98.9 ± 0.106 ^b	100.3 ± 0.158	99.7 ± 0.156	99.2 ± 0.209	98.4 ± 0.20	99.0 ± 0.206	99.9 ± 0.204	100.4 ± 0.156
NAPAP	18	125	99.0 ± 0.108	100.3 ± 0.175	100.8 ± 0.144	100.2 ± 0.118	99.1 ± 0.127	99.9 ± 0.191	100.8 ± 0.163	100.4 ± 0.106
NAPAP	8	100	99.4 ± 0.173	100.7 ± 0.265	100.4 ± 0.244	99.5 ± 0.290	99.7 ± 0.226	100.3 ± 0.314	100.8 ± 0.219	100.3 ± 0.162
SAM	18	150	99.0 ± 0.132	100.6 ± 0.181	99.1 ± 0.160	99.4 ± 0.191	100.9 ± 0.188	100.9 ± 0.148	100.7 ± 0.172	100.6 ± 0.150
SAM	18	125	99.0 ± 0.134	100.4 ± 0.120	100.4 ± 0.148	100.2 ± 0.141	101.1 ± 0.113	101.0 ± 0.115	100.7 ± 0.103	100.7 ± 0.153
SAM	9	100	99.3 ± 0.173	100.5 ± 0.183	100.6 ± 0.240	100.9 ± 0.160	101.4 ± 0.236	100.7 ± 0.310	100.9 ± 0.316	100.6 ± 0.200
ASA	19	150	98.7 ± 0.151	100.1 ± 0.165	101.0 ± 0.153	100.7 ± 0.133	99.3 ± 0.158	99.1 ± 0.185	99.1 ± 0.217	99.3 ± 0.176
ASA	16	125	98.9 ± 0.090	100.5 ± 0.162	101.3 ± 0.157	100.9 ± 0.162	99.6 ± 0.207	99.6 ± 0.110	99.7 ± 0.137	99.9 ± 0.125
ASA	10	100	99.0 ± 0.167	99.9 ± 0.224	100.3 ± 0.177	100.1 ± 0.186	99.5 ± 0.215	99.4 ± 0.158	99.4 ± 0.126	99.3 ± 0.234
Fevered controls	19	Tested with 150 mg./Kg. animals	99.0 ± 0.110	100.4 ± 0.192	101.4 ± 0.190	101.6 ± 0.183	101.4 ± 0.151	101.1 ± 0.165	100.9 ± 0.176	100.7 ± 0.172
Fevered controls	19	Tested with 125 mg./Kg. animals	99.1 ± 0.135	100.3 ± 0.119	101.4 ± 0.158	101.8 ± 0.126	101.6 ± 0.126	101.4 ± 0.133	101.1 ± 0.153	101.2 ± 0.135
Fevered controls	7	Tested with 100 mg./Kg. animals	99.4 ± 0.181	100.2 ± 0.377	100.8 ± 0.460	100.9 ± 0.479	100.5 ± 0.384	100.6 ± 0.260	100.0 ± 0.309	99.9 ± 0.286

^a Rat—Oral Administration ^b S.E. = s/\sqrt{N}

It was observed that SAM was the least potent, most rapid in onset, and had the briefest duration of antipyretic activity of the three compounds tested (Figs. 1, 2, 3). NAPAP differed from aspirin in that it had a more rapid onset and a briefer duration of action. NAPAP and ASA were equally potent, for 100 mg./Kg. of either compound returned the temperature of the febrile rats to the prefebrile readings (see Table III).

Since toxic manifestations or side effects are always of importance in the final evaluation of a drug, a comparison of the ratio of the minimal neurological deficit dose₅₀ (TD₅₀) to the minimal antipyretic dose (min. ED = the lowest dose which reduced the temperature of febrile rats to approximately normal; TD₅₀/min. ED) reveals: NAPAP = $342/100 = 3.42$; SAM = $82/150 = 0.54$; ASA = $312/100 = 3.12$.

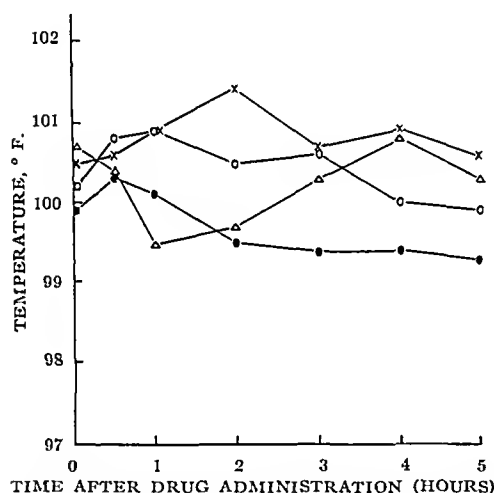


Fig. 1.—Antipyretic activity of 100 mg./Kg. of NAPAP, SAM, and ASA. ○—○ = Fever controls, Δ—Δ = NAPAP, X—X = SAM, ●—● = ASA.

Anticonvulsant Activity.—Doses as large as 25% of the LD₅₀ of NAPAP, SAM, and ASA were ineffective in abolishing the hind limb extensor component of the maximal electroshock seizures in the mouse. There was some indication that NAPAP and SAM prolonged the postictal depression while aspirin was without any apparent effect. This aspect of the convulsive pattern was not explored in greater detail in the present study.

NAPAP, SAM, and ASA in doses as large as 25% of the LD₅₀ did not have significant anti-metrazol activity.

Prolongation of Sleeping Time.—The results of oral administration of NAPAP, ASA, and SAM on the sleeping time of pentobarbitalized mice are presented in Table IV.

In mildly sedating doses, NAPAP significantly prolonged the sleeping time of pentobarbitalized mice while ASA had no significant activity.

Salicylamide had some action in prolonging the sleeping time. However, the dose required to obtain this effect was lethal to 50% of the animals.

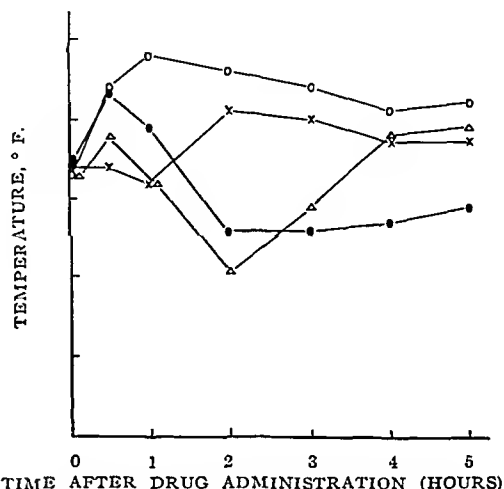


Fig. 2.—Antipyretic activity of 125 mg./Kg. of NAPAP, SAM, and ASA. ○—○ = Fever controls, Δ—Δ = NAPAP, X—X = SAM, ●—● = ASA.

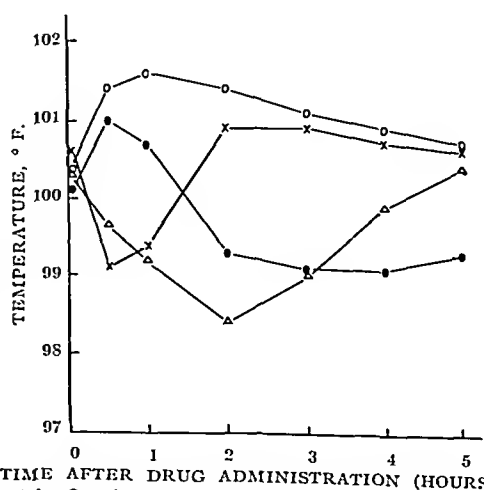


Fig. 3.—Antipyretic activity of 150 mg./Kg. of NAPAP, SAM, and ASA. ○—○ = Fever controls, Δ—Δ = NAPAP, X—X = SAM, ●—● = ASA.

TABLE IV — EFFECT OF ORALLY ADMINISTERED N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYL SALICYLIC ACID ON THE SLEEPING TIME OF PENTOBARBITALIZED MICE

Compound	No Mice	Dose mg./Kg.	LD ₅₀ %	Increase Over Controls, %	P Value	Symptoms Prior to Pentobarbital Na
NAPAP	10	335.0	50	56.75	<0.01	Sedation
NAPAP	10	167.5	25	36.8	>0.02—<0.05	Sedation
NAPAP	10	100.5	15	2.1	>0.05	None
SAM	10	795.0	50	54.6	0.01 ^a	Ataxia, ptosis 5/10 died
SAM	10	397.5	25	13.2	>0.05	Ataxia, ptosis depression
SAM	10	238.5	15	-9.0	>0.05	Ataxia, sedation
ASA	10	395.0	50	-2.35	>0.05	Sensitive to noise
ASA	10	197.5	25	23.27	>0.05	None
ASA	10	118.5	15	1.5	>0.05	None

^a Computed on the basis of the five remaining animals

Respiratory Effects.—The results are summarized in Table V. Respiratory rates and tidal volumes were virtually unchanged by NAPAP and only slightly increased by SAM. In contrast, the most obvious effect of ASA was on respiratory rate. ASA induced a marked increase in rate which became prominent in approximately four hours and increased sharply for the next two hours to a peak of 522% of control. Tidal volume increased *pari passu* with the rate indicating that the primary respiratory effect of ASA was a change in rate. The maximum increase in respiratory exchange at six hours was 532% of control. Tidal volume, respiratory rate, and heart rate increased gradually and moderately for the first three hours. At approximately four hours after ASA all three measurements began to show a sharp increase that extended over the next two hours.

Cardiovascular Effects.—NAPAP produced a small but consistent rise in blood pressure in contrast to SAM and ASA both of which caused a decline in pressure. At the end of six hours dogs receiving NAPAP had the same heart rate as their controls while SAM evoked an 8% and ASA a 77% increase in rate. Rectal temperature was decreased 1.3°C by NAPAP, increased 0.8°C by SAM and increased more than 4°C by ASA. The elevation in temperature induced by ASA began about one and one-half hours after the drug and followed a steadily rising course for the duration of the experiment. NAPAP was the only one of these agents that produced its customary depression of temperature at these high dose levels. Blood pH values did not vary markedly nor correlate well with other observations. The values remained within the normal range for dogs throughout the experimental period.

TABLE V — COMPARATIVE RESPIRATORY AND CARDIOVASCULAR EFFECTS OF N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYL SALICYLIC ACID^a

Hr. After Drug, Inj.	B.P. mm. Hg.	Hr. Rate per Min.	Rectal Temp °C.	Resp. Rate per Min.	Tidal Vol. Control %	Blood pH
N-Acetyl p-aminophenol, 250 mg./Kg. intragastric						
Control	127	146	36.7	23	100	7.40
1	142	162	36.0	30	132	7.44
2	148	153	35.5	26	98	7.50
3	151	154	35.4	20	95	7.46
4	151	147	35.5	19	112	7.44
5	149	144	35.9	24	136	7.46
6	143	148	36.3	22	125	7.44
Salicylamide, 250 mg./Kg. intragastric						
Control	138	160	37.2	30	100	7.42
1	132	153	36.8	27	91	7.39
2	130	151	36.8	27	95	7.46
3	129	157	37.1	29	93	7.46
4	129	154	37.5	31	127	7.42
5	128	162	37.8	33	129	7.30
6	123	173	38.0	34	163	7.40
Acetylsalicylic Acid, 250 mg./Kg. intragastric						
Control	147	144	37.0	18	100	7.46
1	141	154	37.2	27	145	7.42
2	140	170	38.1	29	180	7.46
3	145	180	39.0	33	218	7.43
4	134	195	39.8	41	264	7.42
5	125	222	40.5	69	390	7.38
6	116	253	41.1	94	532	7.41

^a Mean values from 5 dogs on each drug.

(19). In the isolated heart studies the effects of the three drugs at a constant dose of 2 mg. total were compared as to heart rate, myocardial contraction, and coronary flow. ASA produced a definite depression of contraction, a slight reduction in heart rate, and a moderate decrease in coronary flow. SAM elicited a depression of contraction, an increase in coronary flow, and no change in heart rate. NAPAP slightly increased myocardial contraction, caused a moderate increase in coronary flow, and a slight increase in heart rate. These data are summarized in Table VI.

TABLE VI.—MAXIMUM EFFECTS OF N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYLSALICYLIC ACID ON ISOLATED CAT HEARTS^a

Drug	Dose mg.	Amplitude of Beat Control, %	Coronary Flow Control, %	Change in H. R. Beats/Min.
NAPAP	2	101	137	+6
Salicylamide	2	70	191	+2
Acetylsalicylic acid	2	61	84	-4

^a Mean of 5 experiments

DISCUSSION

It was difficult to compare the LD₅₀ values obtained for NAPAP, SAM, and ASA in this laboratory with those of other investigators. The experimental conditions utilized in the different laboratories varied as to animal weight, age, sex, and nutritional state or the experimental background of

the tests were uncertain. Listed in Table VII are the toxicity values and conditions under which the tests were conducted as reported by several authors.

The antipyretic activity of NAPAP (6), SAM (9, 10), and ASA (10) have been reported by several investigators. However, a survey of the literature does not reveal a comparative study of NAPAP, SAM, and ASA under the same experimental conditions.

The results of NAPAP antipyretic activity as reported by Boreus and Sandberg (6) and those found in this laboratory are similar as to time of onset and duration: onset, 0.5–1.0 hour; duration of 3–4 hours.

The tests completed in this laboratory have shown that SAM had a very brief antipyretic activity. The onset of this activity occurred within one-half hour after administration and had completely disappeared within two hours. Furthermore, SAM produced a neurological deficit in doses below the minimum effective antipyretic dose. This has not been reported by other observers in their antipyretic studies. The failure to notice this toxicity might be the result of the physical state of the animals, for during the febrile period the animals remain relatively inactive which tends to mask signs of mild toxicity, e.g., ataxia, central depression. There is a difference in the minimum effective dose of SAM as reported by Bavin (9) and Buller (10) and that determined in this laboratory. Those authors reported that 50 mg./Kg., perorally, had a brief but observable antipyretic effect. The lowest minimal antipyretic dose observed in our studies was 150 mg./Kg. The cause of this difference is unknown; however, it might be attributable to the difference in the fever-producing agent, strain of the rats or the greater predrug temperature produced by their pyretic agent as compared to ours.

TABLE VII.—REPORTED LD₅₀ VALUES FOR N-ACETYL-P-AMINOPHENOL, SALICYLAMIDE, AND ACETYLSALICYLIC ACID

Drug	Species	Nutritional Status, F = fasted	No. of Animals Used per Dose Level	Body Weight, Gm.	LD ₅₀ mg./Kg. with Toxicity Observation Period	Remarks
NAPAP	Rat		2-3	250-350	3200 ^a —48 hr.	Clark (24)
SAM	Mouse	F—18 hr.	10-20	18-25	1590—4 day	Bavin (9)
	Mouse	?	15		1400 ^a —48 hr.	Hart (20)
	Rat	?	15		1400—48 hr.	Hart (20)
	Rat	?	15		1830 ^a —24 hr.	Unchanged at 7 days
	Rat	?		50-80	1200—48 hr.	Ichniowski (22) Male & Female
ASA	Mouse	F	5	18-25	1300—4 day	Wistar Way (25) Male animals used
	Mouse	?	15		1100 ^a —48 hrs.	Bavin (9) Male animals used
	Rat	F—18 hr.	182 total	120-200	1360—7 day	Hart (20) 1 animal/cage
	Rat	?	15		1500—48 hr.	Temp. 78-80° F. Male Wistar rats
	Rat	?	15		1850 ^a —24 hr.	Eagle & Carlson (21) Hart (20)
	Rat	?	15		1100 ^a —7 day	Ichniowski (22)
	Rat	?			1750 ^a —	Ichniowski (22) Grass (23)

^a Approximate LD₅₀ values.

The antipyretic activity of ASA was quite similar to that reported by Buller (10) maximum activity around two hours, duration of action > four hours and a minimum oral antipyretic dose of 100 mg /Kg

These findings correlate well with the effective blood levels of NAPAP, SAM and ASA recently determined by Weikel (26) This author showed that in humans free SAM, the active antipyretic substance, was rapidly conjugated This apparently accounted for its brief duration of antipyretic activity NAPAP differed in that its unbound active form was detectable in the plasma within one hour and was well maintained for approximately five hours ASA took longest to obtain its minimum effect due to the longer time required to obtain peak plasma and tissue levels

It was unexpected that NAPAP was capable of prolonging the sleeping time of pentobarbitalized mice in moderately depressant doses while SAM was inactive except at a dose which was fatal to 1/2 the animals It has been observed by this laboratory and others (27) that SAM is capable of producing hypnosis in nonlethal doses while NAPAP had this activity only in lethal doses Because of the difference in hypnotic activity of the two compounds it would be expected that SAM would be synergistic with pentobarbital more readily than NAPAP The reason for the lack of prolongation by SAM cannot be explained with our present knowledge

The findings of this laboratory on respiratory effects are difficult to compare with the literature since most previous work dealt with intravenous doses of sodium salicylate or with oral doses of ASA in unanesthetized animals The delay of four hours in onset of polypnea with ASA is somewhat surprising in view of other reports indicating that respiratory effects occurred rather promptly (28-32) Although there was a small increase in depth of respiration, the major portion of the increase in respiratory exchange with ASA was due to the increase in rate In agreement with Wright (33) and Graham and Parker (31) it was found that bilateral vagotomy performed during the period of polypnea from ASA would eliminate most of the stimulation and bring respiration to near normal, suggesting the possibility of a central reflex stimulation over vagal afferents

These data indicate clearly that ASA had far more respiratory effects than the other two antipyretics There was no marked change in respiratory rate or tidal volume with NAPAP or SAM

The experimental and clinical literature briefly mentions the effect of ASA on heart rate Graham and Parker (31) gave a rather complete list of clinical symptoms in salicylate poisoning, but did not list tachycardia It is apparent from data reported here that there was a marked tachycardia associated with ASA poisoning, the development of which paralleled that of the respiratory changes Only minor fluctuations in heart rate were found with NAPAP and SAM

References to the direct effect of salicylates on the myocardium are scarce Clark (24) reported that doses of NAPAP from 2 to 25 mg had little effect on contraction or heart rate in isolated rabbit hearts, although it did produce a significant increase in coronary flow Data obtained in cat hearts confirm Clark's report for NAPAP and establish ASA

as being the most depressant to the myocardium with SAM next The actions of ASA and NAPAP are contrasted by the fact that the former produced a definite depression while the latter caused a mild increase in all 3 parameters studied (Table VI) The greater myocardial toxicity of ASA was illustrated by the death of two out of five isolated heart preparations from a 2 mg dose Coronary flow increased to 137% of control with NAPAP without important changes in heart rate or myocardial contraction Interpretation of the increased flow with SAM was complicated by the myocardial depression which accompanied it From these data it would appear that there is little danger of myocardial effects from NAPAP while cardiac depression must be considered as a possibility with toxic amounts of SAM and ASA

SUMMARY

1 Acute toxicity studies on the three antipyretic agents, N acetyl *p* aminophenol (NAPAP), salicylamide (SAM), and acetylsalicylic acid (ASA) revealed that NAPAP was the least toxic (LD₅₀) in rats and guinea pigs while SAM exhibited the least toxicity in mice

2 A neurological deficit (TD₅₀) was produced by SAM at lower doses than NAPAP and acetylsalicylic acid (ASA) in rats and mice

3 All three compounds produce an initial CNS depression Only NAPAP failed to produce convulsions at higher dose levels

4 NAPAP and ASA were approximately equivalent in potency and more effective than SAM as antipyretic agents

5 The antipyresis produced by NAPAP was more rapid in onset than that of ASA but did not have as long a duration of action

6 Consideration of the minimum neurological dose₅₀/effective antipyretic dose indicated that NAPAP and ASA had a wider margin of safety than SAM.

7 NAPAP prolonged the sleeping time of pentobarbitalized mice in nonlethal doses In contrast, SAM was effective only in doses which killed 50% of the animals ASA had no demonstrable effect on sleeping time

8 NAPAP, SAM, and ASA were ineffective as anticonvulsants

9 ASA produced tachycardia, pyrexia, marked polypnea, and an increase in tidal volume of over 500% of control

10 NAPAP and SAM induced little change in blood pH, tidal volume, respiratory rate, or heart rate

11 Myocardial depression was seen with ASA and SAM, but not with NAPAP

12 NAPAP produced a moderate increase in coronary flow without a significant change in force of beat or heart rate

REFERENCES

- (1) Erganian, J. A., Forges, G. B., and Case, D. M., *J Pediatr*, 30, 129(1947)
- (2) Vening, R. M., et al, *Brit Med J*, 23, 414(1957)
- (3) Rodman, M. J., *Am Profess Pharmacist*, 23, 129 (1957)
- (4) Erlanson, A. C., *Prac Staff Meetings Mayo Clinic*, 30, 417(1955)
- (5) Dodd, K., *J Kentucky State Med Assoc*, 55, 137 (1957)
- (6) Boreus, Lars Olaf, and Sandberg, I., *Acta Physiol Scand*, 28, 261 (1953)
- (7) Boreus, Lars Olaf, and Sandberg, I., *ibid*, 28, 266 (1953)
- (8) Hart, R., *J Pharmacol Exptl Therap*, 89, 205 (1947)
- (9) Bavin, E. M., McCrae, I. J., Seymour, D. E., and Waterhouse, P. D., *J Pharm and Pharmacol*, 4, 872 (1952)
- (10) Buller, R. N., Miya, T. S., and Carr, C. J., *ibid*, 9, 128(1957)
- (11) Lehman, A. J., et al., *Toad, Drug, Cosmetic Law J*, 10, 679(1955)
- (12) Litchfield, J. T., Jr., and Wilcoxon, F., *J Pharmacol Exptl Therap*, 96, 99(1949)
- (13) Swinyard, E. A., Brown, W. C., and Goodman, L. S., *ibid*, 106, 319(1952)
- (14) Smith, P. K., and Hambourger, W. E., *J Pharmacol Exptl Therap*, 54, 346(1935)
- (15) Reinhard, J. F., *Prac Sac Exptl Biol Med*, 58, 210 (1945)
- (16) Anderson, F. F., *Arch intern Pharmacodynamie*, 94, 460(1953)
- (17) Anderson, F. F., and Craver, B. N., *J Pharmacol Exptl Therap*, 93, 135(1948)
- (18) Chenoweth, M. B., and Koelle, E. S., *J Lab Clin Med*, 31, 600(1946)
- (19) Duke, H. H., "Physiology of Domestic Animals," Comstock, Ithaca N. Y. 1017, p. 17
- (20) Hart, E. R., *J Pharmacol Exptl Therap*, 89, 205 (1947)
- (21) Eagle, E., and Carlson, A. J., *ibid*, 99, 150(1950)
- (22) Ichniowski, C. T., and Hueper, W. C., *THIS JOURNAL*, 35, 226(1946)
- (23) Grass, M., and Greenberg, L. A., "The Salicylates A Critical Bibliographic Review, Monographs of the Institute for the Study of Analgesic and Sedative Drugs, II," New Haven, The Hillhouse Press, 1948, p. 125. Reported by Carnely, D. A., and Ritter, J. A., *J Am Med Assoc*, 160, 1219(1956)
- (24) Clark, B. B., "Symposium on N-acetyl p-aminophenol, Institute for the Study of Analgesic and Sedative Drugs," Elkhart, Ind., 1952, p. 23
- (25) Way, E. L., Takemori, A. E., Smith, G. C., Jr., Anderson, H. H., and Brodie, D. C., *J Pharmacol Exptl Therap*, 108, 450(1953)
- (26) Wenkel, J. H., Jr., *THIS JOURNAL*, 47, 477(1958)
- (27) Berger, I. M., *Prac Sac Exptl Biol Med*, 87, 449 (1954)
- (28) Rapoport, S., and Guest, G. M., *J Clin Invest*, 24, 759(1945)
- (29) Harvie F. H., and Singer, R. B., *Am J Diseases Children*, 89, 149(1955)
- (30) Johnson, C. C., *J Am Med Assoc*, 94, 784(1930)
- (31) Graham, J. D. P., and Parker, W. A., *Quart J Med*, 17, 153 (1948)
- (32) Smith, P. K., *Pharmacol Rev*, 1, 353(1949)
- (33) Wright, S., *J Pharmacol Exptl Therap*, 54, 1(1935)

Amberlite Resin XE-88 as a Tablet Disintegrant*

By N. J. VAN ABBÉ and J. T. REES

The characteristics of Amberlite Resin XE-88 are described and experimental results are given to illustrate its use as a tablet disintegrant. Amberlite XE-88 is effective, in certain cases, at lower levels than are needed with maize starch; the action is relatively unaffected by magnesium stearate or by prolonged storage.

COMPRESSED TABLETS are convenient for the administration of medicaments in correct dosage but, since they are often swallowed whole, it is essential to make sure either that solution occurs rapidly in the digestive juices or that the tablets break up into small fragments. Further, the active constituents need to be made available for absorption at an appropriate time during passage through the alimentary canal.

When the constituents of a tablet are such that rapid solution does not occur, a substance known as a disintegrating agent or disintegrant has to be included in the formula, the tablet then breaks up into relatively fine particles in water or gastric juice. Varieties of starch (maize, potato, arrowroot, rice) are widely used,

though many other potential disintegrants have been described (1-4). Laboratory examination of a variety of substances suggested that ion-exchange resins would have disintegrant properties.

A considerable field of usage for synthetic ion-exchange resins has developed since 1935, however, the property of exchanging cations or anions is not relevant to the present work except from the standpoint of compatibility with other tablet ingredients. The present study was confined to resins already used in medicine, as it was known that the proposed dosages would exhibit negligible pharmacological effects (5-7). The choice was also limited to those resins which were not strongly colored and which were obtainable in a state of fine subdivision.

Of those resins tested, best results were obtained with Amberlite XE-88,¹ the potassium salt of a carboxylic resin.

¹Supplied by Chas. Lennig & Co. Ltd., London

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This was obtainable as a fine, cream colored powder with a very faint odor, the dry powder was almost tasteless though aqueous dispersions were rather bitter. A 10% suspension in water gave a substantially neutral reaction. The loss on drying at 105° for four hours was about 5% and the ash figure approximately 30%. Water and 95% alcohol soluble extractives were below 0.2%, the arsenic and lead limits were 1 p p m and 5 p p m respectively. These characteristics indicated that Amberlite XE 88 was suitable for incorporation into many tablet formulations.

EXPERIMENTAL

Many variables influence the rate of tablet disintegration, Holstins and DeKay (3), in a statistical study, demonstrated a three way interaction between active constituents, binding agent and disintegrant. Other factors are granule size and porosity, the nature and amount of lubricant, and the degree and speed of compression. Investigation into the disintegrant properties of Amberlite Resin XE 88 was necessarily specific to the materials and formula employed and, while some of the conclusions would be of general application, it was not possible to foresee all the circumstances that might apply in practice.

Since disintegration time depended upon the degree of compression, it was anticipated that various disintegrants might be compared by graphical representation of mechanical strengths and disintegration times recorded over a range of pressure adjustments. Thus, Gross and Becker (2) had compared disintegration times by extrapolation to a standard mechanical strength. In a long series of experiments the present authors failed to demonstrate any simple relationship of this kind and eventually chose to compare the disintegration times of tablets compressed within a narrow range of mechanical strength that would be typical for the particular formulations.

Standardized Conditions of Experiment.—(a) Each series of tablet batches was derived from a large quantity of granules, processed as a single batch. The disintegrants were added to separate portions along with the lubricant. (b) Disintegrants were dried to a moisture content not exceeding 6%. (c) All tablets were compressed on a motorized, single punch Manesty Type E Press. (d) Disintegration times were determined by the method of the 1955 Addendum to the British Pharmacopoeia.

Table I records an experiment in which calcium phosphate B P, which is frequently employed as an inert diluent in tablets, was selected as a typical insoluble constituent, it was moist granulated with the aid of a carefully gelatinized 6% starch paste. No. 14 mesh granules were produced, the "fines" being separated on a No. 40 mesh. According to the amount of disintegrant used, an appropriate quantity of "fines" was replaced in the granulation so that all tablets were derived from mixes containing similar proportions of material passing No. 40 mesh. Tablets were compressed at 0.5 Gm theoretical weight and $7/16$ inch diameter.

TABLE I—COMPARISON OF AMBERLITE RESIN XE 88 AND MAIZE STARCH IN CALCIUM PHOSPHATE TABLETS

Batch No. ^a	Disintegrant	% w/w ^c	Monsanto Hardness, Kg. ^b	Disintegration Time ^d , Sec
1	None		6.0	>3600
2	Maize Starch	3.0	6.6	>3600
3	Maize Starch	6.0	7.0	1550
4	Maize Starch	9.0	5.9	527
5	Maize Starch	12.0	6.0	307
6	Amberlite XE-88	1.5	6.7	>3600
7	Amberlite XE-88	3.0	6.6	1162
8	Amberlite XE-88	6.0	6.9	152
9	Amberlite XE-88	12.0	5.7	21

^a 1 experimental batches of 0.5 Gm tablets of calcium phosphate B P.

^b Hardness and disintegration results are given as the mean derived from two batches. Hardness figures represent the average of 20 readings. Disintegration times are the average of 6 readings taken at time of manufacture.

^c Magnesium stearate 0.25% w/w (throughout).

^d Guided disks used.

The disintegration times were determined on batches of tablets compressed within a Monsanto "Hardness" range of 5.5–7.2 Kg. An exact numerical expression of the relative efficiencies of maize starch and Amberlite XE-88 could not be derived from these results, but comparable disintegration times were obtained when these substances were used in the ratio of approximately 2:3:1.

In the experiment recorded in Table II, phenobarbitone B P (0.032 Gm per tablet) was formulated to yield tablets of 0.065 Gm theoretical weight, compressed at $7/16$ inch diameter. Lactose was used as diluent and 10% gelatin mucilage was employed to produce granules at No. 16 mesh. The "fines" were not removed.

Table III shows the results obtained with varying levels of lubricant. With maize starch, the disintegration time rose abruptly at high levels of lubricant, when Amberlite XE-88 was used, there was no pronounced increase. It is also noteworthy that relatively firm tablets could still be produced on the light duty press, when high levels of magnesium stearate were used in conjunction with Amberlite XE 88 as disintegrant.

The tablets of phenobarbitone were subjected to a storage test under laboratory and "tropical" conditions for twelve weeks. Specimen tubes were both cotton plugged (to allow free passage of moisture vapor) and fitted with waxed corks (to prevent moisture vapor transmission). Pharmacopoeial assays for phenobarbitone performed before and after storage revealed no significant change in any of the tests.

Table III shows that there were no marked changes in disintegration times in any of the sets of conditions.

DISCUSSION

The properties of Amberlite Resin XE 88 which have emerged from the experimental work recorded above, may be considered as follows:

TABLE II—COMPARISON OF AMBERLITE RESIN XE-88 AND MAIZE STARCH IN PHENOBARBITONE TABLETS

Batch No. ^a	Disintegrant	mg /Tab	Magnesium Stearate, mg/Tab	Loss in Wt., Gm. ^b	Disintegration Time, Sec. ^c
1	Maize Starch	6.5	0.45	0.82	85
2	Maize Starch	6.5	0.95	0.86	120
3	Maize Starch	6.5	2.25	0.89	765
4	Amberlite XE-88	2.0	0.45	0.65	55
5	Amberlite XE-88	2.0	0.95	0.42	70
6	Amberlite XE-88	2.0	2.25	0.44	135
7	Amberlite XE-88	4.0	0.45	0.70	45
8	Amberlite XE-88	4.0	0.95	0.48	55
9	Amberlite XE-88	4.0	2.25	0.45	75
10	Amberlite XE-88	8.0	0.45	0.82	55
11	Amberlite XE-88	8.0	0.95	0.62	50
12	Amberlite XE-88	8.0	2.25	0.68	60

^a Experimental batches of 0.065-Gm tablets of phenobarbitone B.P., 0.032 Gm per tablet^b Determined by shaking test (9). A high level of mechanical strength is indicated by a low reading in this test^c Taken at time of manufactureTABLE III—EFFECT OF STORAGE ON DISINTEGRATION TIME^a

Disintegrant	mg /Tab	Magnesium Stearate mg/Tab	Disintegration Time, Sec.			
			After 12 Weeks			
			Initially	Laboratory ^b Cotton-plugged ^d	Waxed Cork ^c	"Tropical" ^c Cotton-plugged ^d Waxed Cork ^c
Maize starch	6.5	0.45	85	60	80	150
		0.95	120	80	120	210
		2.25	765	660	550	570
Amberlite XE-88	4.0	0.45	45	25	55	80
		0.95	55	35	55	90
		2.25	75	35	55	95

^a Storage test results on some of the batches of phenobarbitone tablets shown in Table II^b Normal laboratory conditions (dark cupboard)^c Cabinet maintained at 37° 90% R.H. for 18 hrs. daily, temperature falling to ambient for 6 hrs. and R.H. rising to 100% for short period^d Glass specimen tubes 3 in. x 1 in., with absorbent cotton plugs^e Glass specimen tubes, 3 in. x 1 in., with externally paraffin-waxed corks

In the presence of a considerable proportion of water-insoluble constituents, the inclusion of 5–15% w/w of Amberlite Resin XE-88 yielded tablets having disintegration times well within the acceptable limits but of adequate mechanical strength.

These properties as a disintegrant did not seem to be unduly sensitive to the presence of a hydrophobic lubricant such as magnesium stearate. No marked increase in disintegration time occurred during storage.

The literature showed that Amberlite Resin XE-88 would not have any pronounced physiological action in the dose levels considered and was certainly nontoxic.

Except that exchange of cations might be anticipated in certain instances, Amberlite XE-88 would be chemically compatible with the usual therapeutic agents used in tabletting.

There was no significant effect upon the appearance, odor or taste of the tablets, either initially or after storage, and the requisite proportions did not unduly increase the size.

While these general conclusions are supported by the evidence contained in this paper, it would be imprudent to expect the same results in all circumstances; likewise, it would be incorrect to attempt a quantitative comparison with conventional disintegrants, such as the starches, for general application. However, it does seem that Amberlite Resin XE-88 may be effective in a lower percentage than maize starch and its efficacy is likely to be maintained even when a high proportion of hydrophobic

lubricant is present. This suggests that it might be particularly useful in compositions with a severe tendency to adhere to the punches of a tablet press.

It is possible to offer an explanation of the disintegrant activity of resins such as Amberlite XE-88, by reference to the fact that swelling often occurs when they become hydrated. Many substances exhibit a similar swelling characteristic but do not function satisfactorily as disintegrants. It is suggested that an essential feature of a tablet disintegrant is that, in the swollen state and at temperatures up to 37°, it must not be readily soluble or act as an adhesive; it has often been noted that gums have functioned poorly in this connection probably because, when hydrated, they tended to stick the particles of a tablet together. Ion-exchange resins, while having a swelling effect, do not dissolve or have any adhesive tendency.

REFERENCES

- (1) Berry, H. and Ridout, C. W., *J. Pharm. and Pharmacol.*, **2**, 619 (1950).
- (2) Gross, H. M., and Becker, C. H., *This Journal*, **41**, 157 (1952).
- (3) Holstius, E. A., and DeKay, H. G., *ibid.*, **41**, 505 (1952).
- (4) Firouzabadian, A., and Huyck, C. L., *ibid.*, **43**, 248 (1954).
- (5) Morton, T., *Lancet*, **4**, 825 (1951).
- (6) Barker, R. A., Mackay, I. R., and Evans, B. M., *ibid.*, **2**, 758 (1951).
- (7) Segal, H. L., et al., *Gastroenterology*, **8**, 199 (1947).
- (8) Burlinson, H., and Pickering, C., *J. Pharm. and Pharmacol.*, **2**, 630 (1950).
- (9) Webster, A. R., and Van Abbé, N. J., *ibid.*, **7**, 8 (1955).

Study of Stability of Sulfadiazine Sodium Injection III.*

An Investigation of Degradation Products of Sulfadiazine Sodium

By CHARLES J. SWARTZ† and JOHN AUTIAN‡

Solutions of sulfadiazine sodium undergo discoloration and precipitation on prolonged storage. In this study, the nature of the coloration and precipitation has been investigated utilizing infrared and ultraviolet spectroscopic data together with chemical procedures. The coloration was believed to be composed of sulfanilic-type intermediate oxidation products. The precipitate was ascertained to be principally sulfadiazine contaminated with trace quantities of oxidized materials. Experimental evidence is presented to support the above assumptions concerning these degradation products.

IN PREVIOUS WORK (1, 2), Hom and Autian studied the solubility of sulfadiazine sodium as a function of pH in several solvent systems as well as studying the stability of these formulations. The primary products of deterioration, detected by the coloration and precipitation which form on long standing in sulfadiazine sodium injections, were still unknown.

Numerous investigators (3-5) have studied oxidized or other degraded materials from various sulfonamide solutions but little information concerning sulfadiazine sodium was included.

It was furthermore felt that a quantitative study of the deterioration might shed further light on the process which has been concluded to be one of oxidation(3). Therefore, a study employing five distinct measurements of activity was initiated on samples stored in diffused light at room temperature (20-24°) and those stored at $60.0 \pm 0.1^\circ$ in an oven. This study extended over a one hundred day period.

EXPERIMENTAL

Investigation of Precipitate.—Precipitated material from the deteriorated ampuls was separated by filtration and subsequently washed with several

portions of distilled water. The precipitate was then dried in an oven at 80°. Melting point determination showed a range of 250-254°¹ compared to sulfadiazine which melted at 255-256°.¹ This material was then subjected to infrared spectroscopy employing the "potassium bromide disk" technique which is particularly applicable to the study of minute quantities of insoluble solids. Results of this study are demonstrated in Figs. 1 and 2. Ultraviolet spectra were also prepared in the range of 215 to 320 m μ on samples containing 5.0 mg. of the precipitate dissolved in one liter of a buffered aqueous solution (pH 7.0). A spectrogram was taken in order to check for the possible presence of heavy metal contaminants in the precipitate. It was felt that the metal might be carried over in trace amounts to the refined drug from the manufacturing processes. A Bausch and Lomb Littrow Spectrograph was utilized for this analysis.

Separation and Identification of Colored Material.—The intense brown color present in the solutions was isolated employing a Soxhlet extractor. The solutions were prepared for extraction by filtration, followed by subsequent evaporation to dryness in a forced draft oven at 80°. The resulting solid was stored *in vacuo* over phosphorus pentoxide in a desiccator. This highly pigmented residue was subjected to a continuous extraction by absolute methanol using the Soxhlet equipment and the alcohol then evaporated. The oily product was then returned to the Soxhlet for further extraction with ether. The product obtained at this point was a deep yellow-colored oil which was not immediately crystallizable. Peak absorbances for this oily material were demonstrated in the ultraviolet and visible spectra at 260 m μ and 530 m μ respectively. An infrared spectrum was obtained from the oil, by preparing a smear from which the spectrum was recorded (Fig. 3). After a number of weeks of storage in a refrigerator, the appearance of some fine crystals in the oil was observed.

One Hundred Day Study of Degradation.—A stability study of 1% solutions of sulfadiazine sodium in a borate buffer system varying in pH from 8.5 to 10.0, at increments of 0.5 pH units, was initiated.

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† Abstracted in part from a dissertation submitted by Charles J. Swartz to the Graduate School of the University of Maryland, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, August 1957.

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¹ Uncorrected temperatures.

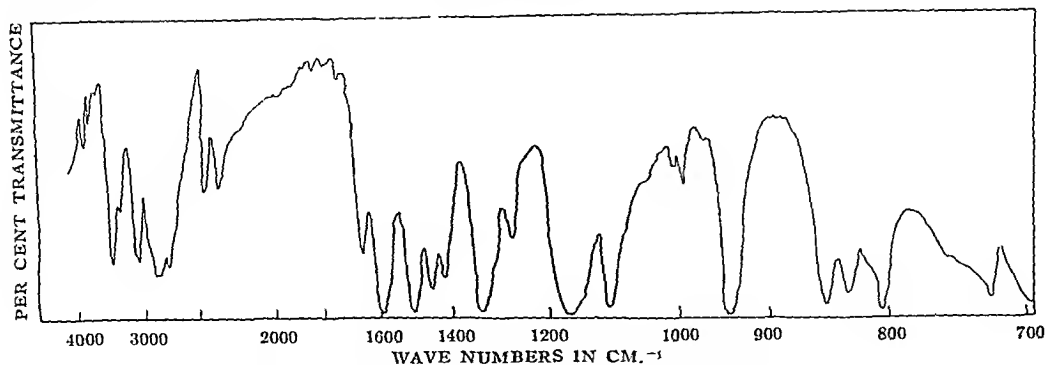


Fig. 1.—Infrared absorption spectrum of sulfadiazine.

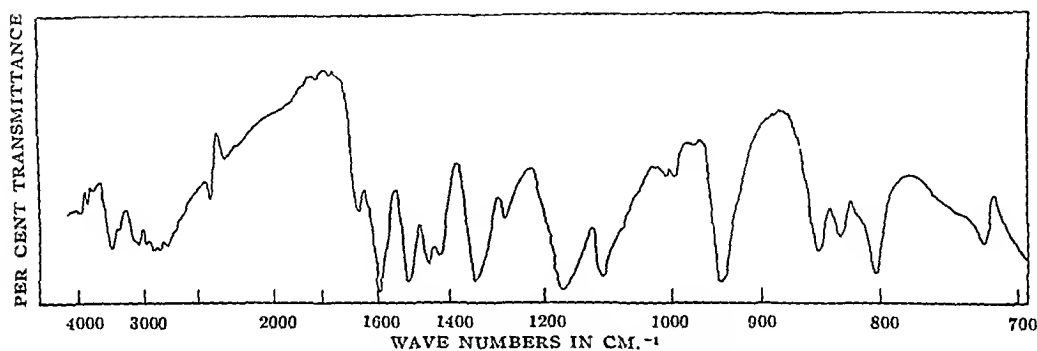


Fig. 2.—Infrared absorption spectrum of precipitate from solution.

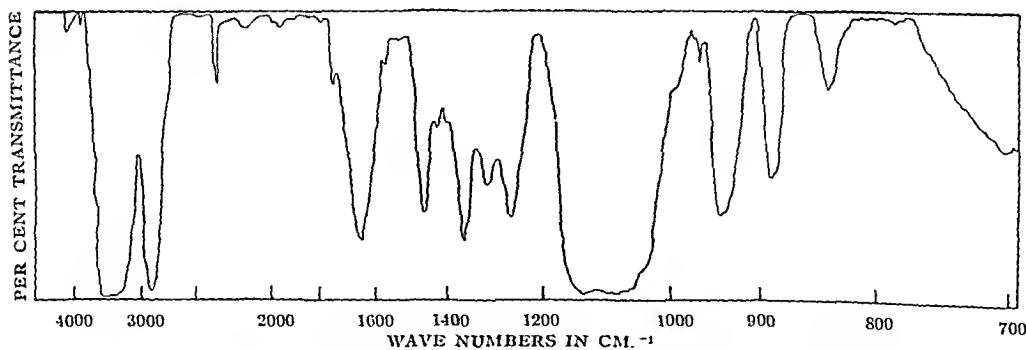


Fig. 3.—Infrared absorption spectrum of yellow oil extracted from degraded solutions.

The samples were placed in 20-ml. clear glass ampuls Type 2 and sealed in the presence of air. One-half of the ampuls were kept at room temperature (20–24°) in diffused light, while the other half were stored in an oven at $60.0 \pm 0.1^\circ$.

At ten-day intervals, the samples were analyzed

by three different procedures. The official nitrite assay for sulfadiazine sodium (6) was employed to detect the benzylamino group. A nonaqueous procedure for the determination of the SO_2NH group developed by Fritz and Keen (7) was adapted and utilized as the second method of analysis. A colori-

metric procedure was employed as a third quantitative technique and was based upon the work of Shepard (8). This method depends upon the reaction of sulfadiazine with 2-thiobarbituric acid to form an intensely colored complex specific for the aminopyrimidine moiety. Hydrogen ion concentrations were obtained by the use of a Beckman Model H2 Glass Electrode pH Meter. Per cent transmission of the samples was determined and the color compared to that of freshly prepared solutions at 520 $m\mu$ on the Beckman Model C Colorimeter (Fig. 4).

Results of this study at 60° are summarized in Table I. At room temperature, there was no measurable degradation after one hundred days.

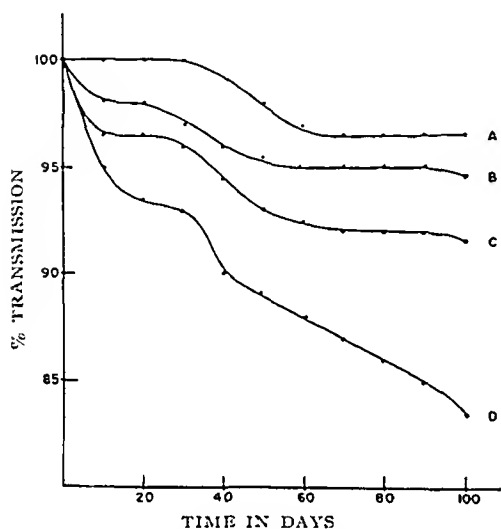
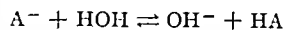


Fig 4—Appearance of color in ampuls of sulfadiazine-sodium (1%) at various pH's stored at 60°. A = pH 10.0, B = pH 9.5, C = pH 9.0, D = pH 8.5.

DISCUSSION

Careful examination of Fig. 2 shows that it differs little from the spectra of pure sulfadiazine (Fig. 1). In addition, examination of ultraviolet data gives further credence to the above observation. The spectrophotometric evidence indicates that the

precipitate is composed of at least 99% sulfadiazine. This precipitation of the free acid is not unusual for salts of weak acids under the conditions of higher concentrations. Even though the hydrolysis equilibrium:



greatly favors the salt form, a small amount of the insoluble acid will be present in solutions of high concentration and will appear as a precipitate. A minute quantity of the colored oxidation products are separated with the sulfadiazine possibly due to an adsorption phenomenon. The depressed and extended melting range of the precipitate substantiates this statement. The spectrogram was completely negative in regards to metallic contaminants in the sulfadiazine powder.

Examination of the infrared spectrum of the extracted oil indicates that it consists of intermediate oxidation products which have already been cleaved from the pyrimidine portion of the molecule. In the region of 800, 1000, and 1500 cm^{-1} , the absence or very minimum appearance of absorption peaks shows that the pyrimidine moiety is no longer present. On the other hand, the spectrum most resembles sulfanilamide in character. There are thirteen absorption peaks in the spectrum of the oil which are identical with those found for sulfanilamide. Maxima at 1250, 1450, and 1735 cm^{-1} which do not correspond to sulfanilamide configuration approximate the maxima observed in sulfanilic acid at these points. The presence of only one absorption maximum, at 260 $m\mu$, in the ultraviolet spectrum also demonstrates the disappearance of the pyrimidine ring in the molecule. These experiments would seem to indicate that the oil is composed chiefly of a mixture of sulfanilic-type compounds which are, themselves, in the process of undergoing further oxidation. Careful analysis of the spectra points to the possible formation of quinoid-like products. Further work, however, is necessary to verify these assumptions.

The numerous analytical procedures demonstrated that, although a color was formed in solutions of sulfadiazine sodium, the degradation in all cases was less than 2% at the end of 100 days storage. Solutions at the higher pH's showed less coloration and at pH 10, the deterioration of ampuls stored at 60° was negligible. No precipitation was noted in any of the ampuls probably because of their dilute nature. Although it has been demonstrated above that the pyrimidine fragment of the sulfadiazine sodium has been cleaved in the early stages of oxidation, loss of this portion of the molecule was not detected in the differential assays.

TABLE I.—STABILITY STUDY OF 1% W/V SULFADIAZINE SODIUM SOLUTIONS STORED FOR 100 DAYS AT 60.0 ± 0.1°

pH		Transmission, %		U.S.P. Assay ^a		Nonaqueous Assay ^a		Colorimetric Assay ^a	
Init.	End	Init.	End	Init.	End	Init.	End	Init.	End
8.50	8.50	100.0	83.5	0.101	0.099	0.101	0.098	0.097	0.096
9.00	9.00	100.0	91.5	0.101	0.099	0.100	0.098	0.097	0.096
9.45	9.45	100.0	94.5	0.100	0.099	0.099	0.098	0.096	0.095
10.00	10.00	100.0	96.5	0.098	0.098	0.097	0.097	0.095	0.095

^a Assay results stated in grams sulfadiazine sodium/10 ml. sample.

It was felt that the colorimetric assay was not sufficiently reproducible to indicate this small loss in potency.

SUMMARY AND CONCLUSIONS

This study has shown that the precipitate which forms in solutions of sulfadiazine sodium on standing is substantially crystalline sulfadiazine with trace quantities of oxidized materials. The oxidation products, extracted as a yellow oil, were postulated to be sulfanilic-type compounds which undergo further oxidation to colored compounds with a quinoid structure.

Analytical procedures demonstrated that degradation of the sulfadiazine sodium in ampuls stored at 60° for 100 days was less than 2% in all quantitative procedures employed.

REFERENCES

- (1) Hom, F. S., and Autian, J., *THIS JOURNAL*, 4S, 608 (1956).
- (2) Hom, F. S., and Autian, J., *Bull. Am. Soc. Hosp. Pharmacists*, 14, 177 (1957).
- (3) James, G. V., *Biochem. J.*, 34, 636 (1940).
- (4) Hamilton, W. F., *et al.*, *THIS JOURNAL*, 33, 142 (1944).
- (5) Whittet, T. D., *Pharm. J.*, 16S, 309 (1950).
- (6) "United States Pharmacopeia," 15th Rev., Mack Publishing Co., Easton, Pa., 1955, p. 691.
- (7) Fritz, J. S., and Keen, R. T., *Anal. Chem.*, 24, 308 (1952).
- (8) Shepard, R. G., *ibid.*, 20, 1150 (1948).

Metabolism of Labeled Acetate in *Bryophyllum Calycinum* Salisb.*

By HERBERT LIEBERMAN,† JOHN E. CHRISTIAN, and
EGIL RAMSTAD

AN UNUSUAL FEATURE of the acid metabolism in *Bryophyllum* is the reported accumulation of isocitric acid in the leaves. Pueher (1) found 50 per cent of the total acidity in *Bryophyllum calycinum* Salisb. to consist of isocitric acid, and Bonner and Bonner (2) stated that approximately 94 per cent of the total acidity in *Bryophyllum crenatum* Salisb. is due to isocitric acid.

An accumulation of isocitric acid above the concentration of citric acid is contrary to the equilibrium constant of the enzymatic reaction: citrate \rightleftharpoons isocitrate. Aconitase, the enzyme mediating this transformation, establishes a mixture of citric, D-isocitric and *cis*-aconitic acids in the proportions of 80, 16, and 4 respectively (3).

Stutz and Burris (4) offered an explanation for the higher concentration of isocitric acid than citric acid in *Bryophyllum calycinum*, stating that, if a tricarboxylic acid cycle is operative in this plant, the cycle is "sluggish." A slow rate of reaction could be due to an inhibition of the aconitase or be the result of the presence of a small amount of this enzyme. But a "sluggish" operation of the tricarboxylic acid cycle cannot rationally explain why there is more isocitric acid than

citric acid in this plant unless isocitric acid is formed prior to citric acid.

The authors of this paper considered the possibility of the existence of a mechanism for the formation of isocitric acid by a route different from that of Krebs' tricarboxylic acid cycle (5). Glyoxylic or glycolic acid was thought to condense with succinic acid to give isocitric acid directly, but such a mechanism could not be found operative in *Bryophyllum calycinum*. However, information appeared in the literature during the course of the present investigation stating that certain bacteria effect a split of citric acid into fragments C2 and C4 (6-12). A similar cleavage of isocitric acid by bacteria was also reported (13). This cleavage does not involve the participation of coenzyme A and has as its products succinic and glyoxylic acids.

In an attempt to establish whether the Krebs' tricarboxylic acid cycle is operative or not in *Bryophyllum calycinum* a comparative study of the metabolism of C¹⁴H₃COOH in the leaves of this plant and in tomato was undertaken.

EXPERIMENTAL

Material and Sample Size.—The leaves of *Bryophyllum calycinum* and tomato were harvested only on bright, sunny days, usually between 3 and 4 p.m., and used immediately thereafter. In order to obtain uniform samples eight medium-sized

* Received September 12, 1957, from the Research Laboratories of the Purdue University School of Pharmacy, Lafayette, Ind.

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leaves from the same clone were placed on top of each other and discs cut with a cork borer number 3 from the middle of the blades, adjacent to each side of the midrib.

Inhibitors.—Respiratory studies in the Warburg apparatus were undertaken to determine the concentration of enzyme-inhibitor which must be added under the chosen experimental conditions to stop respiration in the leaves of *Bryophyllum calycinum* and tomato. Complete inhibition of respiration of *Bryophyllum* and tomato leaves was obtained by imbibition of the leaf with *M*/3 malonate or *M*/30 fluoroacetate. Four conditions were tested in each of the two plants in the $C^{14}H_3COOH$ experiments, namely: (a) no inhibitor, (b) *M*/3 malonic acid, (c) *M*/30 fluoroacetic acid, and (d) both *M*/3 malonic and *M*/30 fluoroacetic acid.

Exposure.—The apparatus employed for vacuum infiltration has been described in a previous paper (5). The plant disks used were pierced with a straight pin and placed into a 2-ml. test tube so that all disks were bathed completely by the imbibition fluid (5). One-half ml. of fluid completely covered the eight disks.

The disks were quickly imbibed with $2-C^{14}$ acetic acid and allowed to metabolize in complete darkness for fifteen minutes in one series, and for two hours in another series of experiments.

Imbibition of $2-C^{14}$ Acetic Acid.¹—The solutions used for the vacuum infiltration of *Bryophyllum* and of tomato contained 50 microcuries of sodium acetate to which were added 2 drops of 1*N* HCl. The acid solution was brought to the one-half ml. mark with water.

Preparation of Autoradiograms.—The techniques employed for imbibition, extracation and spotting have been described previously (5). All samples were run in duplicate, and four samples were run for each type of solution.

The alcohol-soluble residue was suspended in 0.3 ml. of water. From each suspension two 50- λ samples of the supernatant liquid were taken. The samples were spotted in the exact center of a Whatman No. 1 filter paper that had been cut previously to a square, 38 cm. by 38 cm. The diameter of the point of application never exceeded 8 mm. Only the quadrant of the paper into which the radioactive metabolites separated was cut out. Four of these quadrants were taped to a cardboard and placed on the film. The contact time with the film was six to eight months.

Identification of the Compounds.—The identities of the metabolites represented in the darkened areas on the autoradiograms were established by comparison of the metabolites' locations with the locations of known substances similarly chromatographed, and, after elution of the spots, by cochromatographing two-dimensionally the labeled substances with authentic substances.

Generally, the activity of the radioactive spots on any one of the chromatograms was only about 6 counts per minute above background when counted with a Q-gas counter. In order to identify the labeled products, identical spots from 15 sheets were combined. Identical spots were combined only

from chromatograms which had been prepared from experiments with one type of imbibition fluid.

RESULTS

Figures 1-8 show examples of the autoradiograms from the two-hour runs, essentially identical to those from the fifteen minute runs.

DISCUSSION

The findings show that imbibed $2-C^{14}$ acetate is not converted to citric or isocitric acid in leaves of *Bryophyllum*. This holds true whether the experiments were performed with or without the use of malonic and/or fluoroacetic acid as inhibitors. Succinate is the most highly labeled product in *Bryophyllum* (Fig. 1-4), and pyruvic, oxalacetic, and fumaric acids also are labeled relatively highly. When fluoroacetic acid is used as an inhibitor, labeled citric acid is not formed in *Bryophyllum* leaves (Fig. 3).

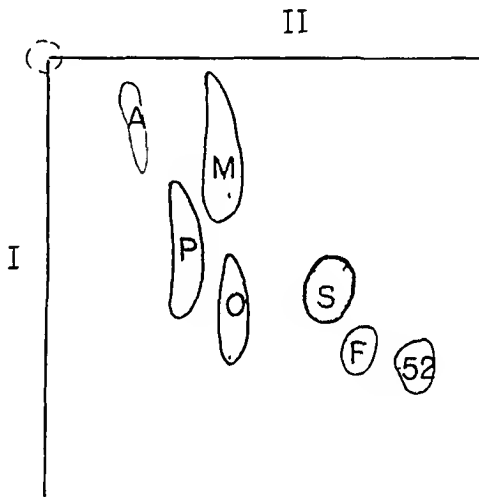


Fig. 1.—Acetate *Bryophyllum* no inhibitor 2 hours. Abbreviations—A, aspartic acid; M, malic acid; P, pyruvic acid; O, oxalacetic acid; S, succinic acid; F, fumaric acid, and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetate acid-water (4-1-1).

Figures 5-8 illustrate the metabolism of $2-C^{14}$ acetate in the control plant, tomato. In this plant succinic acid also appears as the first and the most heavily labeled substance in the experiments with or without enzyme inhibitors (malonic acid, and malonic and fluoroacetic acids together). Citric acid is the most heavily labeled compound produced in the presence of fluoroacetic acid (Fig. 7). Citric acid also appears labeled in tomato, although to a lesser extent, in noninhibited runs with $2-C^{14}$ acetate, but labeled citric acid is not formed in the experiments with *Bryophyllum*.

In the experiments with $2-C^{14}$ acetate in tomato, the presence of labeled citric and malic acids, the

¹ The $2-C^{14}$ acetic acid used in this work was synthesized by G. F. Collins and E. Ramstad (14). The specific activity was calculated from the available data (14) as approximately 15.3 microcuries per mg. of sodium $2-C^{14}$ acetate.

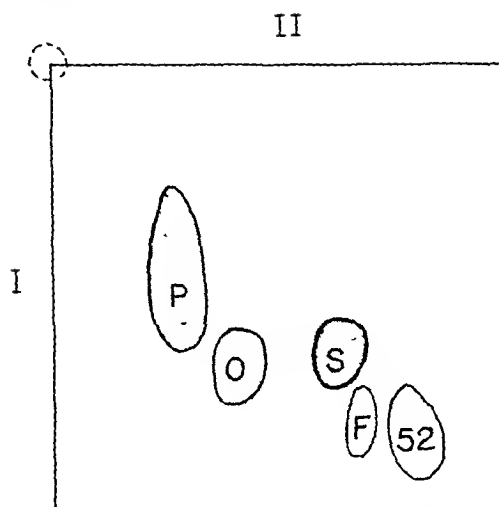


Fig. 2.—Acetate *Bryophyllum* 2 hours malonate. Abbreviations—P, pyruvic acid; O, oxalacetic acid; S, succinic acid; F, fumaric acid; and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetate acid-water (4-1-1).

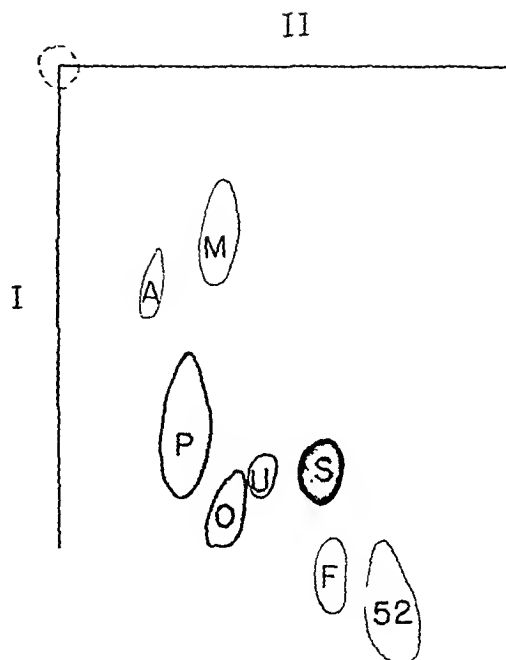


Fig. 4.—Acetate *Bryophyllum* 2 hours fluoroacetate and malonate. Abbreviations—M, malic acid; A, aspartic acid; P, pyruvic acid; O, oxalacetic acid; U, unknown acid; S, succinic acid; F, fumaric acid; and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetic acid-water (4-1-1).

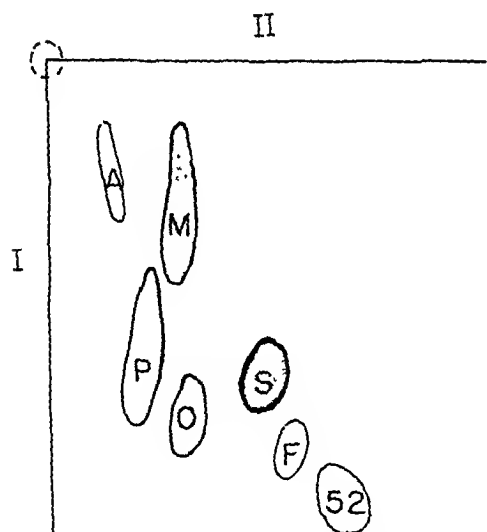


Fig. 3.—Acetate *Bryophyllum* 2 hours, fluoroacetate. Abbreviations—A, aspartic acid; M, malic acid; P, pyruvic acid; O, oxalacetic acid; S, succinic acid; F, fumaric acid; and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetic acid-water (4-1-1).

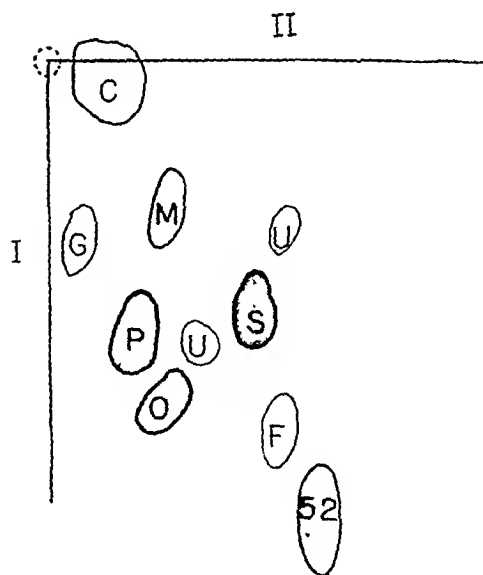


Fig. 5.—Acetate tomato no inhibitor 2 hours. Abbreviations—C, citric acid; G, glutamic acid; M, malic acid; P, pyruvic acid; O, oxalacetic acid; U, unknown acid; S, succinic acid; F, fumaric acid; and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetic acid-water (4-1-1).

increase in labeling of citric acid in the presence of fluoroacetic acid, the formation of labeled glutamic acid (biogenetically related to α -ketoglutaric acid) are findings consistent with the existence of an operating tricarboxylic acid cycle in this plant. In the experiments with 2- C^{14} acetate in *Bryophyllum*, however, the complete absence of labeled citric and isocitric acids, both in inhibited and in non-inhibited runs, the presence of strongly labeled

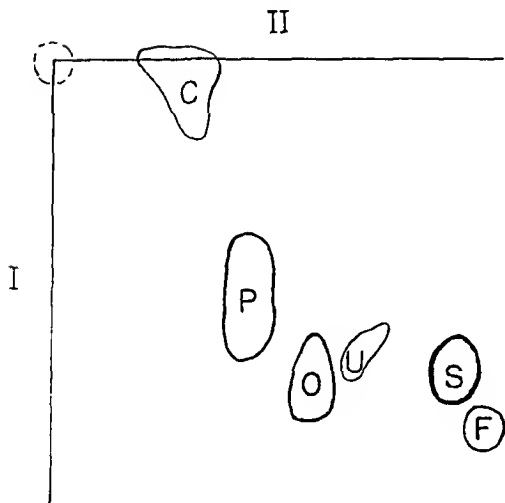


Fig. 6—Acetate tomato 2 hours malonate. Abbreviations—C, citric acid, P, pyruvic acid, O, oxalacetic acid, U, unknown acid, S, succinic acid, and F, fumaric acid. Solvent systems—I, water-saturated phenol, II, butanol-acetic acid-water (4-1-1).

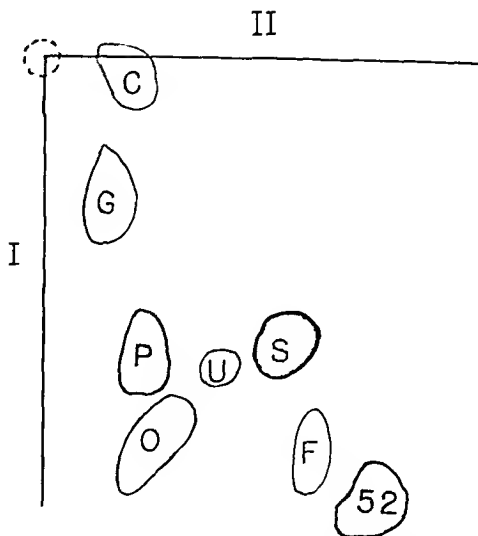


Fig. 8—Acetate tomato 2 hours fluoroacetate and malonate. Abbreviations—C, citric acid; G, glutamic acid, P, pyruvic acid; O, oxalacetic acid; U, unknown acid; S, succinic acid; F, fumaric acid, and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetic acid-water (4-1-1).

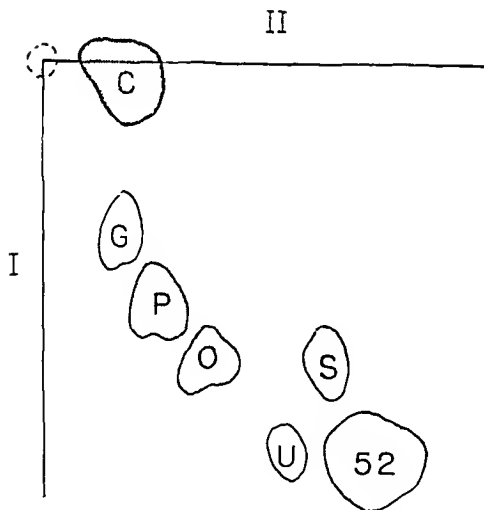


Fig. 7.—Acetate tomato 2 hours fluoroacetate. Abbreviations—C, citric acid, G, glutamic acid, P, pyruvic acid, O, oxalacetic acid, S, succinic acid, U, unknown acid, and 52, acid "52." Solvent systems—I, water-saturated phenol; II, butanol-acetic acid-water (4-1-1).

succinic and also of labeled pyruvic, oxalacetic, and fumaric acids point to the possible existence of an oxidative pathway of acetate that does not involve the tricarboxylic acids.

REFERENCES

- (1) Pucher, G. W., *J. Biol. Chem.*, **145**, 511 (1912).
- (2) Bonner, W., and Bonner, J., *Am. J. Botany*, **35**, 113 (1918).
- (3) Johnson, W. A., *Biochem. J.*, **33**, 1046 (1939).
- (4) Stutz, R. E., and Burris, R. H., *Plant Physiol.*, **26**, 226 (1951).
- (5) Lieberman, H., Ramstad, E., and Christian, J., *This Journal*, **45**, 295 (1956).
- (6) Dagley, S., and Dawes, E. A., *Nature*, **172**, 345 (1953).
- (7) Ajl, S. J., *J. Bacteriol.*, **59**, 499 (1950).
- (8) Campbell, J. J. R., Smith, R. A., and Eagles, B. A., *Biochim. et Biophys. Acta*, **11**, 591 (1953).
- (9) Wheat, R. W., and Ajl, S. J., *Arch. Biochem. Biophys.*, **49**, 7 (1954).
- (10) Sadakawa, T., and Takahashi, K., *Symposia on Enzyme Chem. (Japan)*, **9**, 55 (1954), *Chem. Abstr.*, **48**, 7690 (1951).
- (11) Takahashi, H., *ibid.*, **9**, 61 (1954), *Chem. Abstr.*, **48**, 7690 (1951).
- (12) Wheat, R. W., Wong, D. T. O., and Ajl, S. J., *J. Bacteriol.*, **68**, 19 (1954).
- (13) Smith, R. A., Campbell, J. J. R., and Gunsalus, J. G., *Am. Chem. Soc., Abstr. of Papers*, 126th Meeting, Sept. 1951, p. 75.
- (14) Collins, G. F., Master Thesis, 1952, Purdue University, West Lafayette, Ind.

An Electrolytic Servo-System and the Study of Oxidative Phosphorylation*

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A variety of drugs markedly influence mitochondrial oxidative phosphorylation and other enzyme catalyzed electron exchange reactions. The electrolytic servo-system described is useful in the study of these reactions and the effect of drugs thereon. The servo-system functions to maintain a mediator, transferring electrons between an enzyme system and a metallic electrode, at a constant predetermined potential. The utility of the instrument is demonstrated in a study of the phosphate-uptake associated with the oxidation of α -ketoglutaric acid by rat liver mitochondria, with ferro-/ferricyanide serving as mediator. The selectivity of ferricyanide as an electron acceptor is demonstrated in a study involving the use of Antimycin A.¹ The use of the instrument as a low current potentiostat is also presented.

DURING the last ten years, studies in our laboratory (1, 2), and elsewhere (3), have indicated that certain chemical agents and drugs markedly influence the rate and efficiency of oxidative phosphorylation, the process occurring in mitochondria whereby the oxygen-consuming electron transport reactions are coupled with the formation of high-energy phosphate compounds. Various agents interrupt electron transport at different sites on the electron transport chain; others serve only to uncouple the associated phosphorylation.

Our knowledge of this phase of drug action could be increased significantly if we had an effective means to study the phosphate uptake associated with selected segments of the total electron transport chain. The simple use of oxidation-reduction couples has not been successful as a means of studying the oxidative phosphorylation occurring in selected segments of the transport chain. When used in the concentrations necessary to permit them to serve as the ultimate donor or final acceptor of electrons, most couples have a deleterious effect on the phosphate uptake.

Previous studies in this laboratory (4, 5) have demonstrated the feasibility of using a smooth platinum electrode, together with an electromotively active mediator, to serve as a donor or acceptor of electrons in enzyme catalyzed oxidation and reduction reactions. In these studies, largely unpublished, a smooth platinum electrode was inserted into a modified Warburg vessel and connected through a side-arm to a source of E. M. F. in series with a variable resistance. The circuit was completed by the use of a suitable half-cell in a second Warburg vessel joined to the first cell by a short agar-KCl bridge. A standard calomel half-cell connected to the bridge by a T arrangement permitted measurement by a potentiometer of the potential on the platinum electrode when serving either as a cathode or anode. With no applied E. M. F., the platinum electrode indicated the potential of the electromotively active component (mediator) in the vessel. The inclusion of an ammeter and a voltmeter as in a conventional electrolysis circuit (6) permitted measurement of current and applied E. M. F.

In one group of studies (4) with the platinum serving as an anode, the platinum-containing vessel was charged with a mitochondrial preparation (1) and the necessary components to study oxidative phosphorylation under anaerobic conditions. With a ferro-/ferricyanide couple in catalytic concentrations, serving as the transporter of electrons from the enzyme system to the electrode, the number of faradays consumed during a twenty-five minute period was equal to the equivalents of oxygen used in separate runs under aerobic conditions, while the phosphate uptake was one-third that observed under aerobic conditions. Under aerobic conditions with the platinum serving as cathode and with 2-

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We gratefully appreciated the help given by Drs. F. M. Goyan and K. H. Lee in several phases of the work. Drs. W. K. McEwen and James Buehler worked on earlier modifications of the instrument and contributed significantly to the background essential to the development of the present instrument.

methyl-1,4-naphthoquinone serving as the transporter of electrons from platinum to the enzyme system, but with no substrate, the current matched the measured oxygen uptake and the phosphate uptake was significant (5).

In these studies, the potential of the *mediator* was maintained approximately constant by regulating manually the applied voltage so that the rate of transformation of the mediator by the electrode balanced the rate of the reverse transformation by the enzyme system.

It seemed possible that the usefulness of such an electrolytic device, especially for the study of the influence of drugs on the kinetics of enzyme catalyzed oxidations and on the associated phosphate esterification, could be increased measurably by the development of a servo-system which would automatically control the potential of a mediator within a few millivolts of a prefixed value. The present study is concerned with the development and the use of such an instrument. While there are a number of instruments which maintain a constant potential on an electrode, we are not aware of an instrument, such as that reported here, which maintains the oxidation potential of a mediator constant. Two uses of the instrument are presented. The first employs the instrument as a servo-system in the study of oxidative phosphorylation. In the

second, the instrument is used as a potentiostat to study the kinetics of the electrode oxidation of a mediator.

INSTRUMENTAL

Our approach to the problem of developing an electrolytic servo-system is best made clear through reference to the block diagram in Fig. 1, where the several components of the instrument are represented schematically. Compartment A is a reaction vessel which for the purposes of clarification may be viewed as containing an enzyme system under anaerobic conditions, accepting electrons from its substrate and donating electrons to an added electromotively active mediator. Compartment B contains a half-cell which together with the bridge completes the circuit. The larger platinum electrode (*c*) in compartment A is the working electrode which serves to oxidize the mediator when the potential of the mediator, as a result of enzymic action, falls below a predetermined value. The actual potential of the mediator is measured by use of the Electrometer (setting 3) as the potential between the small platinum electrode (*b*) and the standard reference cell (*a*) (both dipping into the reaction mixture in compartment A).

The presetting of the potential at which the mediator is to be maintained by the working electrode, is made by the use of the Battery Input and the Electrometer (setting 1). The difference in potential between that set by the Battery Input and that imposed by the mediator on the null electrode (together with the reference electrode) at any instant

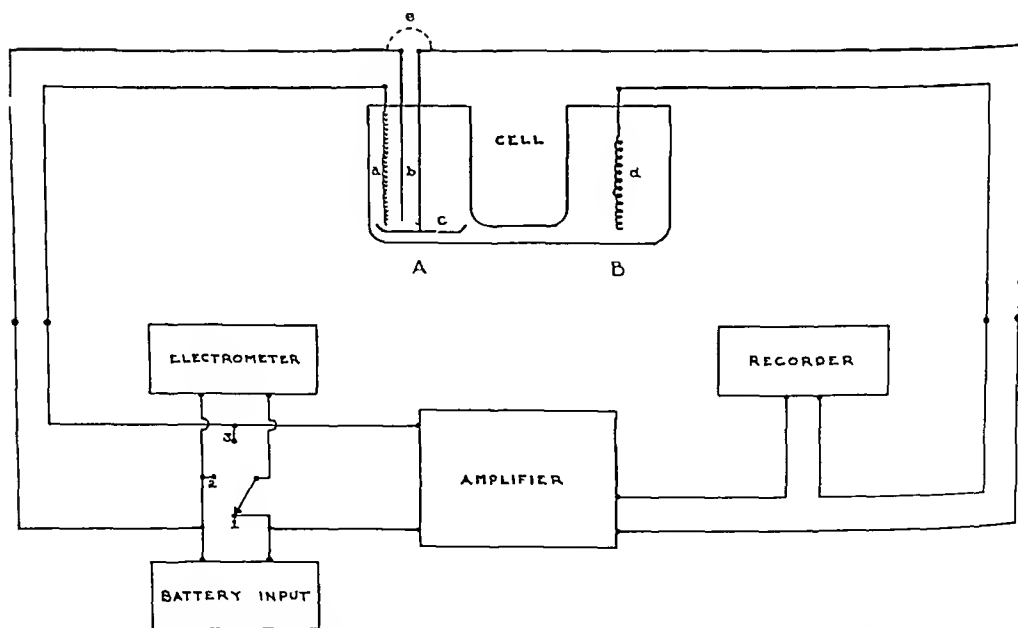


Fig. 1.—Block diagram of the electrolytic servo-system. The labeled components are described in the text.

constitutes the input to the amplifier circuit. When this difference is nil, there is no output from the amplifier and the working electrodes are inoperative. With increasing difference, the output of the amplifier increases proportionately up to a maximum output, at which time the working electrodes are performing at their maximum. The polarity of the amplifier output is always such so as to effect a reduction in the difference in potential between the Battery Input and the sensing electrode system. The Recorder indicated in Fig. 1 served to record the current. The shunt (*c*) shown in Fig. 1 connects the working platinum electrode to both the input and the output of the amplifier and permits the use of the instrument as a potentiostat (see later). Not included in Fig. 1 is the d.-c. power supply for the amplifier.

In the case of enzyme systems capable of reducing oxygen, the mediator may serve as a donor of electrons to the enzyme with the platinum working electrode serving as a cathode.

The Amplifier.—The function of the amplifier is to furnish direct current to the working electrodes in response to information from the sensing electrode system. It was decided that a balanced direct-coupled amplifier would be the simplest and least expensive instrument for this purpose. The circuit is shown in Fig. 2.

To avoid polarization by reducing the current drain on the sensing electrode system, a high-impedance input was provided by using a cathode follower stage whose input resistance is 200 megohms. Maximum input current under the conditions of operation anticipated in this research (0.5-volt maximum input) is 2.5×10^{-9} ampere

Two stages of voltage amplification are provided. Excessive voltages, which would bring about undesired electrolysis of water and other substances, are prevented from appearing on the working electrodes by connecting a pair of twin diodes operating as clipper tubes across the output of the voltage amplifying stages. Two additional cathode follower stages produce the desired direct current at an output impedance of about 700 ohms.

To provide reasonable grid bias voltages while maintaining the input and output connections at a potential near ground, the anodes are supplied from a bus at +150 volts and the cathodes from a bus at -150 volts, both measured with respect to ground.

Since the voltage amplification of the final stage changes with load resistance (R_L), the voltage and current furnished to the working electrode system will depend upon the resistance of the system being electrolyzed. Figure 5 shows the output voltage (V_0) as a function of load resistance (R_L) for three values of input voltage (V_{in}). In practice this variability with load does not cause any difficulty because: primarily the polarity and not the precise value of the output voltage is of importance, and the resistance of the electrode system does not change markedly during the progress of a run.

The overall behavior of the amplifier is shown in Fig. 6, where the output voltage is plotted as a function of the input voltage for three values of load resistance. All three curves were made with the clipping adjustment at the same position, the voltage-limiting effect of which is evident in the figure. It is also evident that the output voltage thus limited is a function of the load resistance.

It is of some interest that though the output po-

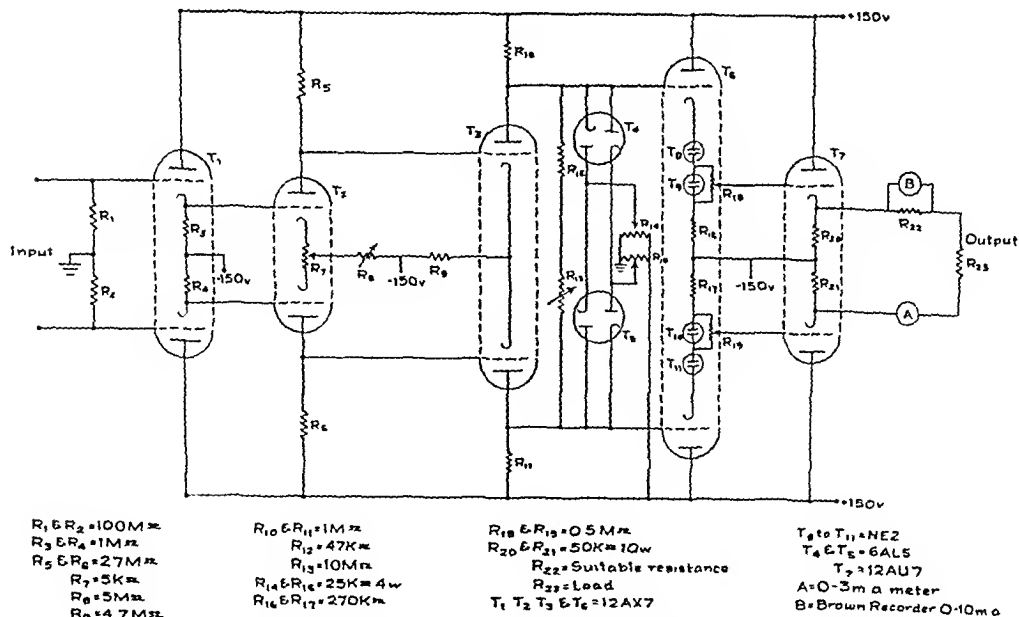


Fig. 2.—Circuit diagram of the amplifier, including current recorder.

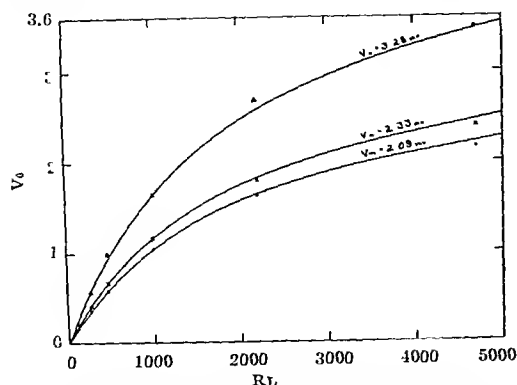


Fig. 5.—The relation between amplifier output in volts (V_o) and load resistance in ohms (R_L) for three values of input in millivolts (V_{in}). The points on the curves are experimental values, while the curves were calculated from an equivalent-circuit analysis using an amplification factor of 15 and a plate resistance of 16,000 ohms for the output tube (12 AU 7), and an over-all gain of 1,620 up to the last stage.

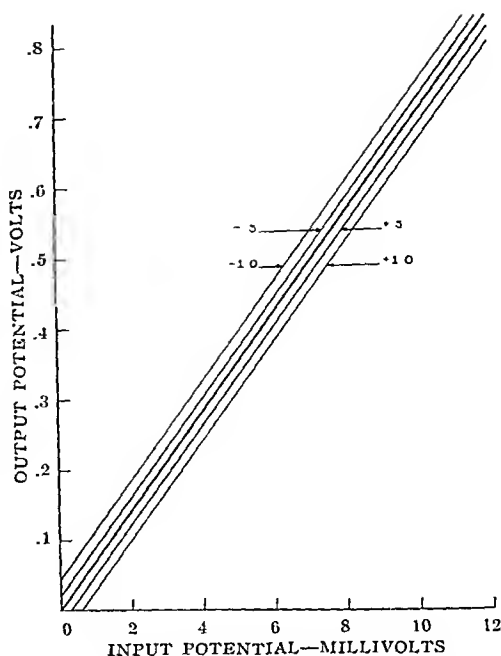


Fig. 7.—Gain characteristics (calculated) of the amplifier with 100-ohm load and no clipping at various values of back E. M. F. (volts). A positive value of back E. M. F. is in opposition to the E. M. F. furnished by the amplifier. The middle curve at zero back E. M. F. corresponds to the lower curve of Fig. 6, but without clipping.

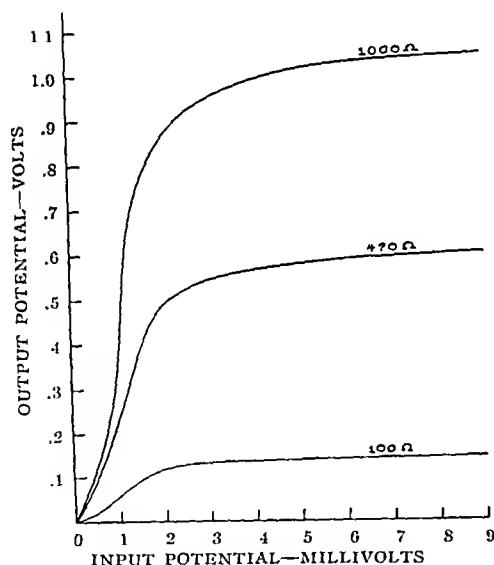


Fig. 6.—Gain characteristics of the amplifier showing the effect of clipping at three values of load resistance. The clipping adjustment was at the same setting in all cases.

figure a positive value of back E. M. F. by convention is in opposition to the output E. M. F..

Power Supply.—The amplifier obtains its operating power from a 300-volt power supply, the circuit of which is shown in Fig. 3. Stability is provided by the use of two 150-volt voltage regulator tubes, which combination also furnishes a center grounded point (see discussion of the voltage requirements above). In order to reduce fluctuations in the out-

put current of the amplifier, it was found necessary to insert a saturable core-transformer stabilizer on the 120-volt line. Further stability may be obtained by separate regulation of the heater voltage supply.

The Battery Input.—The electromotive force which the sensing electrode is to maintain is preset on a common voltage divider powered by two dry cells. The circuit is shown in the bottom center of Fig. 4.

The Electrometer.—It is desirable to be able to measure the electromotive force of the sensing electrode and of the battery input during the progress of a run. Such measurements, to minimize polarization of the sensing electrodes, must be made on an instrument of high input impedance. For this purpose, an electrometer was employed, using a special electrometer tube, Victoreen 5805, in the input stage. The circuit is shown in the upper right hand portion of Fig. 4. The input resistance of the circuit was measured to be 1,000 megohms. Full-scale reading on this instrument (0.5 volt) results in a current consumption of 5×10^{-10} ampere from the system being measured.

The switching arrangement shown in Fig. 4 makes use of a 4-pole 3-position switch. Position 1 connects the battery input voltage to the electrometer; position 2 is a short circuit across the electrometer for zeroing the meter by adjustment of potentiometer R_2 ; position 3 connects the sensing electrode system to the electrometer.

Potentiometer R_1 is used for calibration of the

meter. In our work this potentiometer was set to make 0.5 volt on the input terminals produce a full scale deflection.

The terminals marked "Input" on Fig. 4 are connected to the sensing electrodes, those marked "output" connect to the amplifier.

Electrodes—For a reference electrode, we have had good performance from a Ag-AgCl electrode made according to Lingane (8). The potential of this electrode is +0.197 volt vs. N.H.E. It should be pointed out that with the high input impedance of the electrometer, the calomel half cell used with most pH meters can be used satisfactorily.

The sensing platinum electrode consisted of a short length of platinum wire attached to the side of the reference electrode with the tip of the wire brought close to the sintered glass filter of the reference electrode.

A large Ag-AgCl electrode was used as the half-cell indicated in compartment B in Fig. 1. This non-polarizable cell was made by winding several feet of 0.0179 inch diameter silver wire around 3 mm i.d. glass tubing and bringing the free end up through the bore. An even coating of AgCl was deposited electrolytically.

The working electrode was made of 80 mesh platinum (90%) rhodium (10%) gauze. The center of a square piece was welded to a platinum wire pulled through a short length of 7 mm o.d. capillary tubing and the end of the tube nearest the gauze sealed. The gauze was then moulded into the shape of a bowl and the edges trimmed to yield an area of 5.0 cm². The free end of the tubing was inserted into the chuck of an electric stirrer and the free end of the wire sealed to a slip ring. With this arrangement the platinum working electrode served as a means of stirring the solution in compartment A.

The electrode compartments were made from 10 ml Pyrex beakers joined near the base by a 3 cm section of tubing having 7 mm i.d. The vessel serving as compartment A was provided with a second wall and outlets to serve as a water jacket for purposes of maintaining temperature.

Operation—The several components were connected as indicated in Figs. 1, 2, 3, and 4. The bridge between the compartments A and B was charged as follows: the first several mm nearest compartment A consisted of a 3% Agar 25% saturated KCl, followed by cotton and then a saturated solution of KCl which also constituted the solution in compartment B. Care was taken that the level of fluid in B was in hydrostatic balance with the solution in A. The solution in A, the experimental mixture, contained the enzyme system and the necessary auxiliary components including the mediator. The working electrode was placed in A so it was completely covered by the fluid and so that its rotation would exert the maximum stirring effects. The reference electrode and the sensing platinum electrode as a unit were placed so that the reference electrode and platinum almost touched the stirring platinum electrode.

The amplifier and power supply were turned on several hours before the instrument was used to insure maximum stability. The gain (R_{12}) and level (R_8) potentiometers (see Fig. 2) are set only when the instrument is first prepared for use. The gain control was set at maximum and the level control

such that the voltage on each plate of the first amplifier tube T_2 was between 55–60 volts.

Before each run the balance control (R_7) must be adjusted so that the output (read on the meter) is zero for zero input. The potentiometers R_{13} and R_{19} controlling the grid potentials of the output cathode followers are adjusted so that the output is close to zero relative to chassis ground.

The potentiometers (R_{14} and R_{15}) controlling the clipper diodes are then adjusted symmetrically to limit the output voltage of the working electrodes to the desired value. In the studies on oxidative phosphorylation below, the limitation was such that 1 ma. was delivered to 1000 ohms. In the testing of mediators, also below, the limitation was usually such that 2 ma. were delivered across 1000 ohms.

EXPERIMENTAL APPLICATIONS AND RESULTS

Oxidative Phosphorylation.—The applicability of the electrolytic servo system to the study of enzyme catalyzed reactions was tested in a study of the phosphate uptake associated with the oxidation of substrate in rat liver mitochondria. The P/O value (9) associated with the oxidation of α -keto glutaric acid to succinic acid, in the presence of malonate, was determined under anaerobic conditions using the electrolytic system with the working platinum electrode serving as an anode and with ferro/ferricyanide couple serving as the mediator. Similar trials to serve as controls were carried out in the customary fashion (10) in Warburg vessels, using oxygen as the ultimate electron acceptor.

The rat liver mitochondrial preparation was prepared by the procedure of Dounce, *et al.* (11), with the principal exception that the fluid for the initial homogenization was 0.32 M sucrose and the fluid for washing was 0.25 M, instead of the acidified 0.44 M sucrose.

The reaction mixture amounted to 6.0 ml. in the case of the electrolytic trials and to 3.0 ml. in the case of the Warburg trials. Each ml. of the final reaction mixture contained, in addition to the mitochondrial preparation, the following substances in micromoles: KH_2PO_4 (pH 7.4) 7.5 to 15, KF 9.3, KCl 70, Pabst crystalline ATPNa⁺ 1.0, MgSO_4 8.7, Na malonate 8.0, α -ketoglutarate (pH 7.4) 6.6, and glucose 17.0. Each ml. also contained the equivalent of 0.03 ml. of hexokinase prepared according to Berger, *et al.* (12), (3 a stage).

The mixture for the electrolytic trials contained in addition to the above mentioned components, 0.8 or 1.0 micromole of iron cyanide per ml. added as an approximately equal mixture of $\text{K}_3\text{Fe}(\text{CN})_6$ and $\text{K}_4\text{Fe}(\text{CN})_6$. In some of the trials, for reasons given below, 2,4-dinitrophenol (DNP) was added in concentrations of either 3.3×10^{-5} M or 5.5×10^{-5} M or $1/3$ μg of Antimycin A was added for each ml. of reaction mixture. The amount of mitochondria added to the reaction mixture was estimated by determining the mitochondrial protein in the original suspension by the procedure of Gornall, *et al.* (13), using crystalline bovine serum albumin as the standard. Since these determinations were carried out primarily to permit comparison of the electrolytic runs with the Warburg runs, only comparative data were required and the modification of the procedure recommended by Slater (14) was

not employed. The electrolytic vessels, described previously, after preparation of the bridge, were charged with all components of the reaction mixture, except the mitochondrial preparation, and the several electrodes inserted and connected. For a period of ten minutes, during which time 17° water was circulated through the water jacket, oxygen-free nitrogen was bubbled vigorously through the reaction mixture, which was stirred with the working platinum electrode. At the beginning of this period, the Battery Input was set at 250 mv. positive to the reference Ag-AgCl cell (447 mv. positive to N.H.E.) and the servo-system allowed to adjust the ratio of ferro-/ferricyanide mixture to equal the setting potential. That such a potential was reached could be verified both by the absence of current flow and by the measurement of the potential between the inert platinum and the reference cell.

The cold mitochondrial preparation, immediately prior to adding an aliquot to the reaction vessel, was placed in a chilled Thunberg tube which was then evacuated and flushed with oxygen-free nitrogen several times to remove oxygen. At the time of adding an aliquot of the mitochondrial preparation to the vessels to initiate a trial, the tube delivering nitrogen to the fluid was raised so as to sweep the surface of the liquid free of oxygen. A vigorous stream of nitrogen was maintained throughout a trial. Excessive frothing was observed if the nitrogen was permitted to bubble through the mito-

chondrial-containing reaction mixture. The completeness of the anaerobic conditions established was verified through the polarographic estimation of oxygen in several carefully collected samples of the complete reaction mixture. A temperature of 17° was maintained throughout a trial. After the proper time interval, the trial was terminated by the addition of 0.6 ml. of 50% trichloroacetic acid.

The contents of the vessel were then transferred to a chilled centrifuge tube and the vessel washed with aliquots of 5% trichloroacetic acid to prepare a trichloroacetic acid extract for the determination of the inorganic phosphate by the procedure of Fiske and Subbarow (15). It was established that the low concentration of iron cyanide present in these extracts did not interfere with the estimation of phosphate. The agar plug was removed and the inorganic phosphate determined. The amount of phosphate lost from the main vessel by diffusion against the current-promoted diffusion of anions was small and amounted to 10–16 μ g during a total elapsed time of twenty to twenty-five minutes. Phosphate controls, other than the determination of the inorganic phosphate in the reaction mixture at the start of a trial, were necessitated by a phenomenon observed in the graphs in Fig. 8.

Immediately upon addition of the mitochondria, scanning from right to left in any given trial, the current output rapidly rose to a maximum and fell to a steady-state level. In general, the excursion through the maximum occupied one to two minutes.

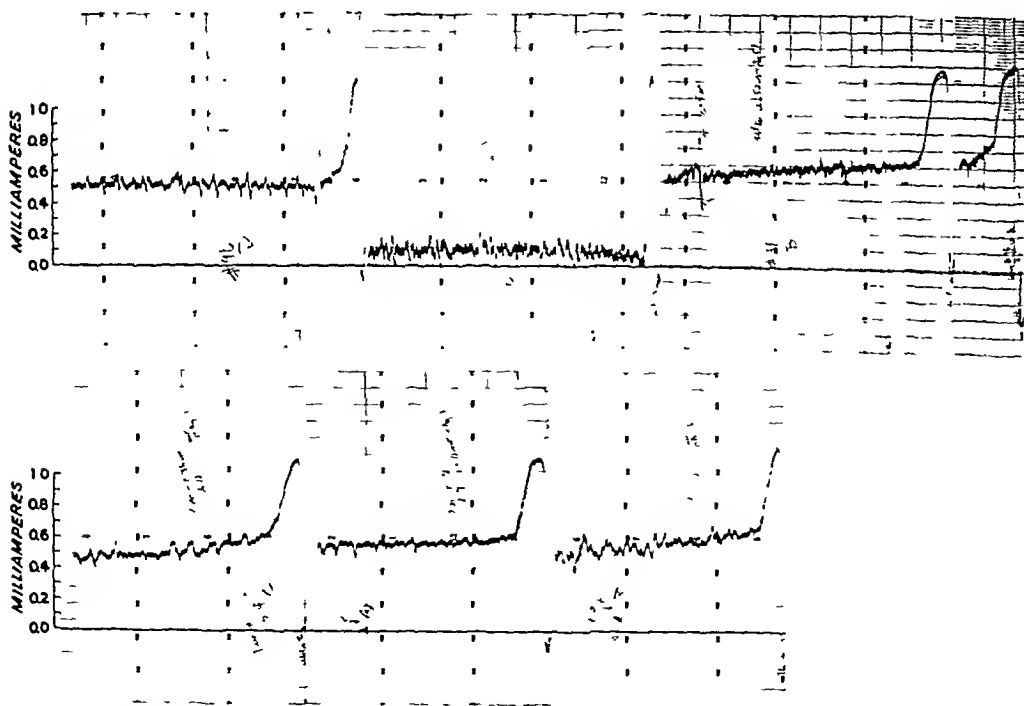


Fig. 8.—Brown recorder tracings of the current output. Each unit on the abscissa time scale represents two minutes. The time scale for each trial runs from right to left. The upper tracing represents three trials and the control (far right) in one experimental series. Both experimental series are described in the text.

This effect could be reproduced by the addition of heat inactivated mitochondria or serum albumin. It has been assumed, though not proven, that the extra current was due to the preferential fixing of the ferriyanide by the protein causing a disturbance in the value of ferro/ferriyanide which served as a large input into the amplifier circuit. The corresponding amplifier output was greater than that which obtained during the steady state level. To obviate the problem caused by the extra current, we regularly terminated either the first or the last trial in a series at the end of four minutes. The value for the inorganic phosphate in this trial was used as the zero time control for the other trials in the series. The measurement of the coulombs consumed in the experimental trials was then taken from the end of the fourth minute after adding the mitochondria. The coulombs consumed were estimated either from the product of the current, as read on the ammeter in series with the Brown Recorder, and the time elapsed from the end of the control period, or by use of a planimeter.

The Warburg vessels were loaded in the cold (0-2°) with 0.3 ml of 50% trichloroacetic acid added to a side arm and with 0.2 ml of 20% KOH added to the center well. All runs were conducted at 17° with two controls and at least two experimental flasks in each trial. Inorganic phosphate was determined in trichloroacetic acid extracts prepared at the end of the eight minute temperature-equilibrium period, in the control, and at the end of an additional fifteen minute period in the experimental flasks.

The time dependent characteristics of the current output of the servo system are presented for two representative runs of three trials each in the graphs in Fig 8. The "noise" level of the output varied from trial to trial, upon occasions being either greater or less than is to be observed in the graphs. It was observed that other voltage measuring equipment in the laboratory, such as the refrigerator unit used to maintain a temperature of 17°, had some effect on the output. It was also observed that the high frequency "noise" was directly connected with the stirring operation, both frequency and amplitude being related to the rate of stirring. We are not clear as to the cause of the low frequency excursion. It is not probable that it was due to a low frequency oscillation in the amplifier which was tested with an oscilloscope. It is possible that closer attention given to electrode design and materials will reduce the variation in output.

In all of the trials presented in Fig 8, the potential on the inert platinum electrodes was maintained

constant at a value within a few millivolts of the setting value. The constancy of the value established that the mitochondrial system was being oxidized as a potential of 450 mv positive to the NHE and that the working platinum electrode was oxidizing the reduced mediator just as rapidly as the oxidized mediator was reduced by the mitochondria. Under these conditions the current output is an accurate index (except for noise) of the rate of oxidation of the mitochondria. In order to bring about the observed rates of oxidation, the total output across the working electrodes, while approximately constant for each trial, varied between 0.3 and 0.4 V for the several trials.

A summary of the results relating the microequivalent of oxygen (Warburg trials) or electrons (electrolytic system) consumed per mg of mitochondrial protein to the microatoms of phosphate uptake is presented in Table 1. The values for the amount of mitochondrial protein per vessel are also presented. It can be observed in Table 1 that both types of runs proceeded at approximately the same velocity. The average "P/O" values associated with the single step oxidation of α ketoglutaric acid amounted to 3.1, with oxygen as electron acceptor, and to 1.9 with the ferro/ferriyanide as the acceptor. The average value of 1.9 for the electrolytic trials was calculated from the results of all trials carried out, except those involving the use of 2,4-dinitrophenol and Antmycin A (below). It should be pointed out that many of the trials, carried out during the early period when we were gaining experience with the operation of the servo system, gave "P/O" values no higher than 1.0-1.4. Nine of the last thirteen trials gave "P/O" values of 2.0 or higher with an average value for the nine of 2.5. There is reason to believe that the higher values are more reliable.

In a previous study (4), evidence was supplied that the phosphate uptake in the electrolytic vessels was enzyme catalyzed and not due to an electrode reaction. To obtain additional evidence on this point, use was made of the fact that 2,4-dinitrophenol uncouples the enzyme phosphorylation as associated with mitochondrial oxidation (16, 17). The lower tracing in Fig 8 is from a three trial run in which the reaction mixture of the middle trial contained $3.3 \times 10^{-5} M$ DNP, in addition to the usual components. The "P/O" value associated with this trial amounted to 1.2 while the values for the "P/O" of the trials on either side, without DNP, amounted to 3.1. In three other trials, involving the use of $5.5 \times 10^{-5} M$ DNP, there was observed an increase in inorganic phosphate amounting to

TABLE 1—SUMMARY OF RESULTS USING WARBURG TECHNIQUE AND ELECTROLYTIC SERVO SYSTEM

Type of Experiment	No of Experiments	No of Trials	mg Mitochondrial Protein Vessel	$\mu\text{eq}/\text{mg}/15 \text{ Min}$	$\mu \text{ atom P}$ $1/2 \times \mu\text{eq}$
Warburg	10	24	16.4(12-21) ^a	0.130(0.098-0.195)	3.1(2.4-3.7)
Electrolytic	15	26	23.3(16-30)	0.125(0.078-0.165)	1.9(1.0-3.1)

^a The numbers in the last three columns represent the average values and the range of values. The results do not include the data from those trials involving the use of either DNP or Antmycin A.

about 2 micromoles per vessel. Since under our experimental conditions the Warburg control trials also showed an increase in inorganic phosphate in the presence of the same concentration of DNP, we did not seek an explanation for the liberation of inorganic phosphate. Clearly, the phosphate uptake observed in the electrolytic trials without DNP is the result of mitochondrial oxidative phosphorylation.

The high value for the "P/O" in the electrolytic trials suggested the use of Antimycin A to test the inference that ferricyanide was constrained to accept electrons from a site or sites in the electron transport chain proximal to the site of action of Antimycin A. The addition of $1\frac{1}{2}$ μ g. Antimycin A per ml. of reaction mixture in three trials in the iron cyanide-mediated oxidations caused a consistent and marked depression of coulombic consumption amounting to 82, 78, and 86% of the control value. The upper tracing in Fig. 8 is from an experimental series in which the middle trial was inhibited by Antimycin A. The coulombic consumption in this trial was but 24% of the value for the control (left). The trial to the right in the same tracing involved the use of 3.3×10^{-5} *M* DNP. There was phosphate liberation in both drug-treated trials. The control gave a "P/O" value of 1.4.

Potentiostat.—While the instrument described has been designed to function as a servo-system to maintain a *mediator at a fixed potential*, with a change in connection of but a single lead, the instrument serves admirably as a low current potentiostat to maintain the *working electrode at a fixed potential*. The change consists in connecting one lead of the amplifier input to the working electrode rather than to the null electrode (see *e* Fig. 1). Under these conditions, the setting of the Battery Input determines the constant voltage.

The use of the instrument as a low current potentiostat is particularly valuable in problems involving the use of mediators and in the study of mediators *per se*. A mediator is valuable only so long as the rate of reaction with the electrode is as fast as or faster than its rate of reaction with the enzyme whose reaction it mediates. The value for the *effective* kinetic constant of the reaction between mediator and the working electrode can easily be determined by the use of the instrument as a potentiostat.

In Fig. 9 and Table II are presented the results of the use of the potentiostat to follow the rate of oxidation of ferrocyanide by the working electrode with the potential maintained constant.

In this trial, 6.0 ml. of 0.001 *M* potassium ferrocyanide in 0.15 *M* potassium chloride was placed in compartment A. The bridge was loaded as described previously. Compartment B with the large Ag-AgCl electrode contained a saturated solution of potassium chloride. The Battery Input was set at 395 mv. positive to the Ag-AgCl reference electrode. During the course of the trial, a vacuum tube voltmeter and the electrometer were used to measure, respectively, the potential between the cathodic Ag-AgCl (*d* Fig. 1) and the reference Ag-AgCl (*a* Fig. 1) electrodes and between the platinum working electrode and the silver reference electrodes.

The numbers on the abscissa of Fig. 9 and listed in Table II under measurement number, indicate the times at which the potential measurements were

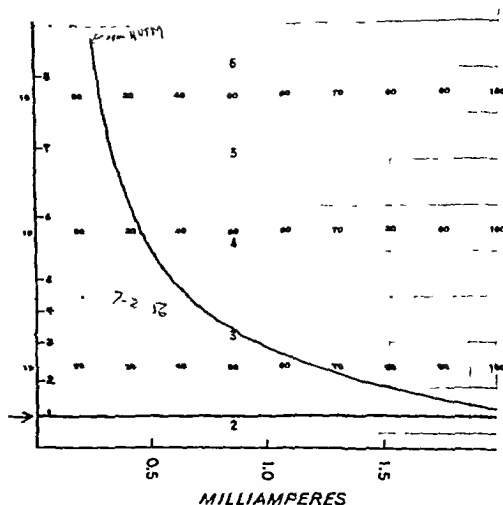


Fig. 9.—The time-dependent current output in the oxidation of ferrocyanide at constant potential. The experimental conditions are described in the text.

TABLE II.—THE ANODIC OXIDATION OF FERROCYNIDE AT CONSTANT POTENTIAL

Measurement Number	Voltage relative reference cell, Millivolts		Current, Milli-amperes	Resistance, Ohms
	Anode	Cathode ^b		
1	380	-220	1.92	110
2	395	-150	1.42	110
3	395	-110	0.94	120
4	395	-80	0.73	110
5	395	-70	0.59	120
6	395	-50	0.43	120
7	395	-40	0.33	120
8	395	-30	0.27	110

^a In calculating the resistances, the values for the boundary potentials have been neglected.

^b This voltage represents the IR drop across the bridge between the two electrode compartments.

taken. The space between the heavy lines on the abscissa scale in Fig. 9 represents two minutes.

In Fig. 9, the arrow indicates the start of the trial. Immediately there was a rapid increase in output with the current exceeding the range of the recording instrument. The value reported in Table II (measurement 1) was taken from an ammeter in series with the recorder. The absence of noise in the tracing of the current output in potentiostat trials was a consistent and understandable finding.

As can be observed in Table II, the potential on the working electrode, after a brief initial period, was maintained constant at a value of 395 mv. It is evident, in comparing Table II and Fig. 9, that associated with the fall in current there is a

concomitant change in potential on the cathodic silver electrode, which is accounted for by the decline in IR. Calculations indicate the resistance was constant at about 100 ohms. The pattern of the potential changes observed in this study has been described by Lingane (17) and need not be considered here.

When the log of the current was plotted against time, a straight line was observed, indicating that with constant voltage on the platinum electrode, ferrieyanide is oxidized according to first order kinetics. It is important to know whether or not the electrode reaction is a single reaction, especially when complex media are involved. The linear relation when observed establishes the fact that the current efficiency for the reaction is 100%. This point was confirmed by the spectrographic analysis (420 $m\mu$) for ferrieyanide.

The specific rate constant for the oxidation (or the reduction) of a mediator calculated from the semilogarithmic plots permits calculation of the rate of the electrode reaction at various concentrations of mediator. Such calculations permit the use of the minimum concentration of mediator. Replication of the trial at several voltage settings furnishes data relative to the influence of voltage on velocity. A single trial takes but a few minutes to complete. It makes little difference for practical purposes whether or not the velocities measured are diffusion limited.

DISCUSSION

The experiments on oxidative phosphorylation, especially those involving the electrolytic system, were not carried out with the goal of establishing the conditions best suited for maximum "P/O" values. It was believed that by using conditions found to be suitable for good values in Warburg runs, evidence would be obtained to establish the usefulness of the electrolytic system in the study of problems in enzyme kinetics.

The electrolytic system, used as a servo system, makes possible the transfer of electrons between an enzyme system and a mediator at fixed potentials. The mediator may serve either as the donor or the acceptor of electrons and need be present only in catalytic amounts. The experimental opportunities offered by these conditions, we believe, are brought out in the results of the studies on oxidative phosphorylation.

The range of "P/O" values associated with the single step oxidation of α ketoglutaric acid, in the Warburg trials, was wider and the average value significantly lower than has subsequently been obtained with essentially the same procedure in this laboratory (18), when no attempt was made to coordinate such runs with the electrolytic runs. However, the higher values of the present data are wholly compatible with a "P/O" value of 4 for the oxidation of α ketoglutarate as observed by Copenhagen and Lardy (10, 19). Likewise the nine best values for the electrolytic runs (with an average "P/O" value of 2.5) are compatible with a "P/O" value of 3.0. Further the average values for all the data from each type of run, when corrected for phosphate leaks either on a proportionate or on an ab-

solute basis, are consonant with the view that the results of the electrolytic runs differ by only one P/O unit from the results of the Warburg runs. In the careful studies by Copenhagen and Lardy (10) wherein greater than stoichiometric amounts of ferrieyanide were used as the ultimate electron acceptor, the "P/O" value for the single step oxidation of α ketoglutaric acid was 1.5 with a range of values between 1.2-1.9, indicating a difference in two P/O units between the values with ferrieyanide and oxygen.

In the electrolytic runs, three microequivalents of ferrieyanide, at a fixed potential, mediated six microequivalents of oxidation, while in the studies of Copenhagen and Lardy, 50 microequivalents of ferrieyanide, at no fixed potential, were associated with only 16 microequivalents of oxidation. With lower concentrations of mediator, at fixed potential, it is reasonable to expect a more selective acceptance of electrons, since each donor site on the mitochondria undoubtedly has a different energy of activation relative to the reaction with ferrieyanide. Support for this expectancy is found in the results using Antimycin A, a highly selective inhibitor blocking the transport of electrons in mitochondria at an intermediate site in the transport chain (20). In the results of Copenhagen and Lardy (10), Antimycin A did not block the reduction of ferrieyanide by mitochondria. It was suggested that ferrieyanide accepted electrons proximal to the block. In our results, Antimycin A was an effective blocking agent, indicating that to a large extent ferrieyanide was constrained to accept electrons at a site distal to the block. Results similar to ours have been reported recently by Pressman (21) under experimental conditions which permitted the use of low concentrations of ferrieyanide (but not at fixed potential). He observed both a high "P/O" value and inhibition by Antimycin A.

Copenhagen and Lardy also observed, with high concentrations of ferrieyanide, at high but changing potential, that the rate of oxidation was greater than with oxygen as the acceptor. In our studies, the rate of oxidation with ferro-/ferrieyanide at an electrode potential of 0.450 was only slightly lower than with oxygen.

The electrolytic system, used as a potentiostat, makes possible the testing of the suitability of mediators for use as donors or acceptors in enzyme studies. In using a mediator as a donor of electrons in a multienzyme system reducing oxygen, an evaluation must be made of the rate of auto oxidation of the mediator. The rate of auto oxidation of a mediator, under the conditions it is to be used in the enzyme runs, can be ascertained with ease through use of the servo system in separate runs on the mediator.

In the developmental stages of these studies it was hoped that the servo system would be valuable in obtaining data relating the rate of an enzyme promoted reaction to the potential of the electron donor or acceptor, both in simple and in multienzyme systems. The estimation of "decomposition" potentials can be used to calculate energies of activation for particular enzyme reactions. However, the noise level of the output of the servo system must be reduced, possibly by electrode design and modification of the amplifier, to achieve these possibilities.

SUMMARY

1. The electrolytic servo-system described serves to maintain the potential of a mediator transferring electrons between an enzyme system and a metallic electrode at a constant value. Accordingly, the mediator need be used only in catalytic concentrations. The low concentration, together with the constancy of potential, permits the mediator to exert a more selective acceptor or donor role in multienzyme oxidation and reductions.

2. The use of the servo-system is demonstrated in a study of the oxidative phosphorylation associated with rat liver mitochondria. The ferricyanide mediated oxidation of α -ketoglutaric acid yields a higher P/O and greater selectivity of action than when the ferricyanide is used in stoichiometric amounts. Studies using Antimycin A support the evidence for the selectivity of electron acceptance by mediator-ferricyanide.

3. The servo-system may be used as a low current potentiostat to study the kinetics of the mediator-electrode interaction.

REFERENCES

- (1) Eiler, J J, and McEwen, W K, *Arch Biochem*, 20, 163(1949)
- (2) Eiler, J J, Krezanoski, J Z, and Lee, K H, *Federation Proc*, 15, 247(1956)
- (3) Hunter, F E, Jr, and Loury, O H, *Pharmacol. Revs*, 8, 89(1956)
- (4) McEwen, W K, and Eiler, J J, *Federation Proc*, 9, 200(1950)
- (5) Eiler, J J, and McEwen, W K, Unpublished
- (6) Lingane, J J, "Electroanalytical Chemistry," Interscience Publishers, Inc, New York, 1953, p 203
- (7) Reich, H J, "Theory and Application of Electron Tubes," 2nd Ed, McGraw-Hill Book Co, New York, 1944, p 166
- (8) Lingane, J J, *loc cit*, p 264
- (9) Hunter, F E, Jr, "A Symposium on Phosphorus Metabolism," Vol 1, The Johns Hopkins Press, Baltimore, Md, 1951, p 297
- (10) Copenhagen, J H, and Lardy, H A, *J Biol Chem*, 195, 225(1952)
- (11) Dounce, A L, Witter, R F, Montv, K J, Pate, S, and Cottone, M A, *J Biophys and Biochem Cytol*, 1, 139(1955)
- (12) Berger, L, Stein, M W, Colowick, S P, and Cori, C F, *J Gen Physiol*, 29, 141(1946)
- (13) Gornall, A G, Bardowill, C J, and David, M M, *J Biol Chem*, 177, 751(1949)
- (14) Cleland, K W, and Slater, E C, *Biochem J*, 53, 547(1953)
- (15) Fiske, C H, and Subbarow, Y, *J Biol Chem*, 81, 629(1929)
- (16) Loomis, W F, and Lipmann, F, *ibid*, 173, 807(1948)
- (17) Lingane, J J, *loc cit*, pp 185-192
- (18) Lee, K H, to be published
- (19) Lardy, H A, and Copenhagen, J H, *Nature*, 174, 231(1954)
- (20) Potter, V R, and Reif, A E, *J Biol Chem*, 194, 287(1952)
- (21) Pressman, B C, *Biochim et Biophys Acta*, 17, 273(1955)

Dispersing Agents in Reaction Media*

By BERNARD ECANOW and DALE W. DOERR

p-Aminobenzoic acid was dispersed in a polyethylene glycol 400 and water solution. The dispersed acid underwent a normal Sandmeyer reaction to give *p*-cyanobenzoic acid in good yields.

DUE to the electrophilic nature of the carboxyl group, *p*-aminobenzoic acid will not form a hydrochloride salt to any extent. It exhibits a relatively low solubility in an aqueous solution of hydrochloric acid. Partly because of its low solubility, *p*-aminobenzoic acid when used to prepare *p*-cyanobenzoic acid, via the normal Sandmeyer reaction gives poor and often erratic results (1-3).

In a procedure (4) using an "inverted" Sandmeyer reaction, the sodium salt of the acid is made. In the resulting solution *p*-aminobenzoic acid lacks stability because of the ease with which the amino group becomes oxidized in basic solutions.

A clear and stable solution was obtained when *p*-aminobenzoic acid was dispersed in a mixture of polyethylene glycol 400 and water. It was found that a normal Sandmeyer reaction occurs satisfactorily in this medium.

Crude *p*-cyanobenzoic acid is a colloidal mass that binds water quite firmly and is usually contaminated with inorganic substances. Recrystallization from water is a very inefficient process (5). The pure *p*-cyanobenzoic acid was

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obtained from the crude product by extracting with ether as described by Ecanow and Gisvold (6).

A partial purification was accomplished by dissolving the crude product, obtained from the Sandmeyer reaction, in polyethylene glycol 400. Upon heating to 100° the water was boiled away from the colloidal acid dispersion. The inorganic contaminants, which are insoluble in polyethylene glycol 400, then precipitated from solution. The pure acid remained in the polyethylene glycol 400 and could not be recovered by practical laboratory methods.

EXPERIMENTAL

Preparation of *p*-Carboxybenzene Diazonium Chloride.—Fourteen grams of *p*-aminobenzoic acid was dissolved in a mixture of 70 ml. of polyethylene glycol 400 and 30 ml. of water contained in a 500-ml. beaker.

Eight grams of sodium nitrite was dissolved, with stirring, in 10 ml. of water, filtered, and then added to the above solution. The combined solution, labeled solution *A*, was cooled in an ice bath. Twenty milliliters of cold concentrated hydrochloric acid was poured into a 500-ml. beaker and 50 Gm. of ice was added, labeled as mixture *B*, and placed in an ice bath.

Solution *A* was slowly added to mixture *B* with constant stirring. During the diazotization reaction, the temperature was maintained between 0° and 5°. After the addition of solution *A* was completed, stirring was continued for ten minutes. One-half gram of urea was then added to eliminate the excess of nitrous acid.

A solution of 4 Gm. of sodium hydroxide dissolved in 10 ml. of water was then added slowly, and with efficient stirring, to neutralize the extra mole of hydrochloric acid. The reaction mixture, now only slightly acidic, was stirred for an additional ten minutes. The resulting mixture contained the *p*-carboxybenzene diazonium chloride as a mixture of suspended crystals and soluble diazonium salt and was labeled as mixture *C*.

Preparation of *p*-Cyanobenzoic Acid.—In an 800-ml. beaker 13.5 Gm. sodium cyanide was dissolved in 60 ml. of distilled water. With exterior cooling from an ice-water bath, 11 Gm. of cuprous cyanide was added in portions with stirring. Then 13.6 Gm. of sodium carbonate monohydrate was added; much of the latter did not dissolve. This was labeled mixture *D*, and cooled to 0° in an ice-salt-water bath.

Mixture *C* was added to mixture *D* in small portions with vigorous stirring. Excessive foaming was controlled by the addition of a few drops of *n*-octanol. After the addition of mixture *C* was completed, stirring was continued for an additional fifteen minutes. This mixture was then removed from the ice-salt-water bath and heated at 100° for two hours with occasional stirring. The cloudy solution was then filtered through a Büchner funnel and the filtrate was concentrated, until the early indication of pellicle formation, by means of an air jet directed onto the surface of the solution. The solution was refrigerated for two days, during which time a mass of crystals of sodium *p*-cyanobenzoate formed. These crystals were collected on a Büchner funnel. The crystals were then dissolved in 100 ml. of water and filtered to remove any insoluble materials. The filtrate was stirred, under a hood, while a slight excess (approximately 15 ml.) of concentrated hydrochloric acid was slowly added. The crude *p*-cyanobenzoic acid that separated was collected on a Büchner funnel and washed with ice water. This crude product was a hydrated colloidal material with a slight green color. The crude *p*-cyanobenzoic acid was recrystallized by the method as described by Ecanow and Gisvold (6). The pure crystalline *p*-cyanobenzoic acid melted at 219° as reported in the literature (2–4, 6). Yields varying from 9.8 Gm. (65%) to 11.6 Gm. (77%) were obtained.

SUMMARY

1. Polyethylene glycol 400 gave a clear and stable dispersion of *p*-aminobenzoic acid in water.
2. *p*-Aminobenzoic acid undergoes a Sandmeyer reaction satisfactorily in a polyethylene glycol 400 and water medium.
3. This method could prove useful for running Sandmeyer reactions on other compounds which cannot be readily solubilized by other means than through a dispersing agent.
4. A partial purification was accomplished by dissolving the crude product, obtained from the Sandmeyer reaction, in polyethylene glycol 400.

REFERENCES

- (1) Sandmeyer, R., *Ber.*, 17, 1633, 2650(1884).
- (2) DiGangi, F. E., and Gisvold, O., *This Journal*, 38, 154(1949).
- (3) Chow, A. W., and Gisvold, O., *ibid.*, 41, 202(1952).
- (4) Miller, R. H., and Gisvold, O., *ibid.*, 42, 367(1953).
- (5) Kilpatrick, M., and Banes, R. D., *J. Am. Chem. Soc.*, 65, 589(1943).
- (6) Ecanow, B., and Gisvold, O., *This Journal*, 46, 315(1957).

The Interaction of Epidermal Protein with Aluminum Salts*

By I. LYON and I. M. KLOTZ

Aluminum ion is bound to human keratinous materials. Aluminum interaction with (guinea pig) epidermis is strongly dependent upon pH. The carboxylate groups of the protein are probably the primary sites of aluminum sorption.

ALUMINUM SALTS are widely used as topical antiperspirants. However, there is little information concerning the interaction of epidermal protein with these salts. The studies reported here were undertaken (a) to determine the order of binding of aluminum to various keratinous materials, (b) to define some of the experimental conditions affecting the binding process, and (c) to determine the sites of the protein adsorbent at which binding occurs.

EXPERIMENTAL

Preparation of Adsorbents.—Human Callus.—The callus (obtained surgically) was washed with Triton X-100, a nonionic surface-active agent, and rinsed in distilled water. It was then extracted with petroleum ether and acetone, and powdered in a ball mill. The powdered callus was treated with a salt solution, 1.5 *M* NaCl or 0.6 *M* $\text{Al}_2(\text{SO}_4)_3$, by immersion for fifteen minutes. Samples of the treated powder were mounted on albumin-surfaced glass slides, washed several times with distilled water, and stained with 8-hydroxyquinoline reagent for aluminum according to a modification of the method described by Glick (1).

Human Skin.—The washing and extraction procedures were performed as described for the callus material. Skin scrapings were then processed, stained, and examined as indicated above.

Human Hair.—A tress of human hair (previously shampooed in soap and water but otherwise untreated) was washed with Triton X-100, thoroughly rinsed in distilled water, and divided into three parts. One part was immersed in 1.5 *M* NaCl solution, another in an aqueous solution of 0.6 *M* $\text{Al}_2(\text{SO}_4)_3$. Immersion time was fifteen minutes. The fibers were then stained with Thioflavin TG, a fluorochrome, washed in distilled water, and air-dried. Hair samples were mounted in paraffin oil and observed under ultraviolet light. From the third part of the tress, cross sections of unstained

NaCl-treated and $\text{Al}_2(\text{SO}_4)_3$ -treated fibers were prepared with the Hardy microtome (DeLaRue, Hyattsville, Md.), stained with the 8-hydroxyquinoline reagent, and examined by ultraviolet light microscopy.

Guinea Pig Epidermis.—Guinea pig skin, obtained from animals sacrificed within one-half hour, was freed of most of its hair and all of the subcutaneous tissues. It was then placed, epidermis up, on a warming table kept at about 50° for five to fifteen minutes. This technique has been shown to facilitate separation of the epidermis from the underlying dermis (2). The epidermis was carefully scraped from the dermis, washed several times in tap water, rinsed in distilled water several times, and stored in the refrigerator in distilled water.

Initial results were not reproducible when epidermal protein (hereinafter referred to as epidermin) samples, prepared as described above, were incubated in an aluminum-containing medium. This lack of reproducibility may have been related to variations in the epidermin. The latter, in turn, may be related to differences among animals due to age, sex, nutritional state, etc. To minimize these possible sources of nonuniformity in the epidermin samples, large amounts of pooled epidermis were lyophilized and mined. Epidermin samples prepared in this way yielded fairly reproducible sorption results, within 3%.

Colorimetric Determination of Aluminum in Solution.—The determination of aluminum used is based upon the formation of a colored complex between aluminum and the ammonium salt of aurintricarboxylic acid (3-5). Measurements were made in a Beckman spectrophotometer at 530 μ in a potassium acetate buffer, pH 3.7, after a three hour color development period.

Amperometric Titration of Chloride.—The titration of chloride in dilute solution was carried out using a AgNO_3 solution and a rotating platinum (indicator) electrode. The method is a modification of that described by Laitinen, *et al.* (6).

Measurement of pH.—Measurements were made with either the Beckman pH meter, Model G, or the Cambridge pH meter, Model R, with a standard No. 1190-80 glass electrode. Since the aluminum salt solutions were unbuffered, there was considerable galvanometer drift and it was difficult to obtain a stable pH reading. To diminish or eliminate the drift, pH determinations were made in the presence of approximately 0.015 *M* added KCl. Hydrogen ion concentrations were determined from the pH meter readings, the ionic strength of the salt solutions, and the corresponding activity coefficients for hydrogen ion.

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Incubation Procedure.—*Preincubation*—Preincubation of the epidermin constituted a pH equilibration prior to incubation with an aluminum solution at the same pH. Seven ml of an HCl solution (pH 3.0–4.0) were added to each of four vials containing 100 mg of epidermin. The vials were preincubated for 15 minutes on an agitator. Aliquots were taken from duplicate vials 1 and 2, as well as from the initial HCl solution, for pH and chloride ion determinations.

Incubation—Two milliliters of AlCl_3 solution (650 μg of aluminum ion per ml) were added to each of duplicate vials 3 and 4. The final concentration of aluminum ion was 144.4 μg per ml. Vials 3 and 4 were placed on the agitator and incubated for fifteen minutes. Aliquots were removed for pH, aluminum ion, and chloride ion determinations. An AlCl_3 solution, designated "144," was made by adding 4 ml of AlCl_3 (650 μg per ml) to 14 ml of the initial HCl solution, and aliquots were taken for determination of pH, aluminum ion, and chloride ion.

CALCULATIONS

Aluminum Sorption Data.—The net change in concentration of aluminum ion may be computed from the concentration change occurring during the incubation period alone, i. e., the difference between the aluminum ion concentration of the "144" solution, and the average concentration of aluminum ions in vials 3 and 4. For example when the aluminum ion concentration¹ of the "144" solution was $6.30 \times 10^{-3} M$, the average concentration of aluminum ions in vials 3 and 4 was $4.45 \times 10^{-3} M$, and the volume of the incubation medium was 9 ml. The moles of Al^{3+} bound by 100 mg epidermin may be computed as follows: $[(6.30 \times 10^{-3} M) - (4.45 \times 10^{-3} M)] (9 \times 10^{-3} L) = 1.66 \times 10^{-5}$. This figure may then be readily converted to 1.66×10^{-4} moles of Al^{3+} bound per Gm epidermin. The results of these computations are assembled in Table I.

Hydrogen Ion Sorption.—*Preincubation*—The sorption of hydrogen ion in this stage may be computed from the difference between the hydrogen ion concentration in the initial HCl solution and the average concentration of H^+ ions in vials 1 and 2 (I). For example, when (H^+) was $4.7 \times 10^{-4} M$ in the original HCl solution, the average concentration in vials 1 and 2 after equilibration was found to be $1.27 \times 10^{-6} M$. Since the volume of the preincubation medium was 7 ml, the moles of H^+ bound per 100 mg epidermin may be computed as follows: $[(4.7 \times 10^{-4} M) - (1.27 \times 10^{-6} M)] (7 \times 10^{-3} L) = 3.28 \times 10^{-6}$. Consequently 3.28×10^{-5} moles of H^+ must be bound per Gm epidermin.

Incubation—Hydrogen ion sorption in this stage may be computed from the difference between (H^+) in the "144" solution and the average concentration of H^+ in vials 3 and 4 (II). The net change in (H^+) due to sorption of aluminum may be calculated from (II) minus (I).

For example, in the experiments in which (H^+) in the "144" solution was $2.9 \times 10^{-4} M$, the average concentration of H^+ in vials 3 and 4 was $9.6 \times$

TABLE I.—EFFECT OF pH ON THE SORPTION OF ALUMINUM SULFATE AND OF ALUMINUM CHLORIDE BY EPIDERMIN

Final pH		Aluminum Sorbed (Moles $\times 10^4$ per Gm Epidermin) ^c
A $\text{Al}_2(\text{SO}_4)_3$		
1	46 ± 0.04^a	0.11 ± 0.27
3	26 ± 0.18^a	0.61 ± 0.21
4	21 ± 0.06^a	1.09 ± 0.07
B AlCl_3		
2	22 ± 0.05^a	0.04 ± 0.04
3	51 ± 0.09^a	1.66 ± 0.05
3	93 ± 0.22^b	1.23 ± 0.04
4	01 ± 0.27^b	1.16 ± 0.03
4	10 ± 0.17^b	1.12 ± 0.04
4	17 ± 0.07^b	0.72 ± 0
4	30 ± 0.07^b	0.29 ± 0

^a Each pH measurement and the corresponding sorption result represent the mean \pm the standard deviation of the mean of 6 replications.

^b Each pH measurement and the corresponding sorption result represent the mean \pm the standard deviation of the mean of 3 replications.

^c The incubation medium contained 144.4 μg of aluminum ion per ml. Each vial contained 100 mg of guinea pig epidermin in a total volume of 9 ml. Adjustment of pH was made with either HCl or NaOH. Incubation time, fifteen minutes, room temperature, approximately 25°.

$10^{-5} M$. The incubation volume was 9 ml. Assuming that no H^+ is produced or removed due to the hydrolysis of aluminum ion,² we may calculate the moles of H^+ bound by 100 mg. epidermin during incubation as follows: $[(2.9 \times 10^{-4} M) - (9.6 \times 10^{-5} M)] (9 \times 10^{-3} L) = 1.75 \times 10^{-6}$. Thus we obtain 1.75×10^{-5} for the moles of H^+ bound per Gm. epidermin.

On the basis of the above simplifying assumption, the moles of H^+ sorbed in preincubation minus the moles H^+ sorbed during incubation equals the moles of H^+ released by aluminum sorption on the epidermin. Thus, $[(3.28 \times 10^{-5}) - (1.75 \times 10^{-5})] = 1.53 \times 10^{-5}$ moles of H^+ released per Gm. epidermin.

Chloride Sorption.—The net change in (Cl^-) may be obtained in a manner analogous to that indicated for hydrogen ion.

Preincubation—The chloride ion concentration in an original HCl solution was $1.94 \times 10^{-3} M$, the average concentration of chloride ions in vials 1 and 2 was $1.79 \times 10^{-3} M$, and the volume of the preincubation medium was 7 ml. Thus, 0.11×10^{-5} moles of Cl^- were bound per 100 mg. epidermin or 1.1×10^{-5} moles of Cl^- were bound per Gm. epidermin.

Incubation—The chloride ion concentration of the "144" solution was $1.680 \times 10^{-2} M$, the average concentration of chloride ions in vials 3 and 4 was $1.613 \times 10^{-2} M$, and the volume of the incubation medium was 9 ml. The Cl^- bound per 100 mg epidermin = 0.060×10^{-4} moles, or 6×10^{-5} moles of Cl^- were bound per Gm. epidermin.

The net moles of Cl^- bound by the epidermin during incubation may now be computed $[(6 \times$

² It is probably an oversimplification to assume that no H^+ is produced or removed by the hydrolysis of aluminum ion. However, the data are insufficient to calculate the concentration of $\text{Al}(\text{OH})^{2+}$ formed by the reaction of Al^{3+} with water.

¹ These calculations are based upon the maximal aluminum sorption which occurred at pH 3.51, with AlCl_3 .

10^{-5} moles Gm.⁻¹) - $(1.1 \times 10^{-5}$ moles Gm.⁻¹)] = 4.9×10^{-5} moles of Cl⁻ bound per Gm. epidermin.

A summary of the above sorption computations will be found in Table 11

the keratinous adsorbent. Thus, aluminum is bound strongly to callus keratin, which under polarized light, appears to be largely amorphous with some areas of low orientation. However, skin

TABLE 11—RELATIONSHIPS AMONG SORPTIONS OF HYDROGEN, CHLORIDE, AND ALUMINUM IONS BY EPIDERMIS

Ion ^a	Initial	Final	Difference
Preincubation			
Hydrogen	3.29×10^{-5}	0.01×10^{-5}	3.28×10^{-5}
Chloride	13.6×10^{-5}	12.5×10^{-5}	1.1×10^{-5}
Incubation			
Hydrogen	2.61×10^{-5}	0.86×10^{-5}	1.75×10^{-5}
Chloride	151.0×10^{-5}	145.0×10^{-5}	6.0×10^{-5}
Aluminum	5.67×10^{-4}	4.01×10^{-4}	1.66×10^{-4}
Net Change in Ionic Binding to Epidermin (Incubation difference minus preincubation difference)			
Hydrogen		1.53×10^{-5} released	
Chloride		4.9×10^{-5} bound	
Aluminum		1.66×10^{-4} bound	
Ionic Binding Ratios			
Aluminum/Hydrogen		$\approx 11^b$	
Aluminum/Chloride		≈ 3	
Chloride/Hydrogen		$\approx 3^b$	

^a Data are expressed as moles per Gm. epidermin

^b It should be noted that these ratios are computed from the sorption of one ion (aluminum or chloride) and the release of another ion (hydrogen)

RESULTS AND DISCUSSION

Aluminum Binding Data

Aluminum Binding to Human Keratin.—Microscopic examination under ultraviolet light showed appreciable amounts of aluminum bound to the keratin particles of callus previously treated with Al₂(SO₄)₃ and then stained with 8-hydroxyquinoline. In similar experiments with keratin scrapings from the dorsum of the hand, lesser amounts of aluminum were bound. Aluminum sorption was observed only after the hand had been washed with lipid solvents. Since no aluminum was bound before washing with solvents, binding may have been hindered by the presence in skin keratin of lipid materials. Of course, solvent treatment could have resulted in some denaturation of the keratin even though it is an insoluble protein.

Intact hair fibers showed no difference in fluorescence (visualized with Thioflavin TG) between aluminum salt-treated and untreated fibers. However, in cross sections of hair fibers stained with 8-hydroxyquinoline very small amounts of aluminum were observed by ultraviolet microscopy. The aluminum was restricted to the cuticular substance of the fibers.

It is thus evident that aluminum is bound to keratin: strongly to callus keratin, less strongly to skin keratin, and least to hair keratin. The amount of aluminum which is bound may be related to the degree of orderliness or close-packed orientation of

keratin, a less ordered material than callus, binds less aluminum than does the latter. Hair, which has a high order of organization and orientation in many areas and offers a high degree of steric hindrance, binds little aluminum, certainly less than callus and skin keratins. Further, in hair, aluminum binding is restricted to the birefringent cuticle.

Aluminum Sorption by Epidermin.—*Time Data.*—These experiments (in triplicate) were carried out at pH 3.5 and approximately 25° to determine the incubation time required to obtain maximal aluminum sorption by the guinea pig epidermal adsorbent. The incubation medium contained 130 µg. of Al³⁺ (as AlCl₃) per ml. Aluminum sorption by epidermin reached a maximum in fifteen minutes or less and remained at that level ($8.46 \pm 0.61 \times 10^{-5}$ moles per Gm. epidermin) throughout an incubation period of 120 minutes.

Aluminum Sulfate Sorption Data.—The relationship between pH of the incubation medium and aluminum sorption from Al₂(SO₄)₃ is shown in Table 1. It may be seen that as the pH of the medium was increased from 1.46 to 4.21 the amount of aluminum bound also increased from 0.11×10^{-4} to 1.09×10^{-4} moles per Gm. epidermin.

Aluminum Chloride Sorption Data.—The relationship between aluminum sorption from AlCl₃ and pH is presented in Table 1. The highest sorption, 1.66×10^{-4} moles per Gm. epidermin, occurred at pH 3.51 with sorption falling off sharply both above (pH 4.30, 0.29×10^{-4} moles per Gm. epidermin)

and below that pH (pII 2.22, 0.04×10^{-4} moles per Gm epidermin)

A comparison of the sorption data obtained with the two aluminum salts (Table I) shows that the pII at which maximum binding occurs is different for each salt. With the *sulfate* 1.09×10^{-4} moles per Gm epidermin were sorbed at pH 4.21. This sorption level may or may not be maximal since the pH range was not extended above pH 4.21. With the *chloride* 1.66×10^{-4} moles per Gm epidermin were sorbed at pII 3.51. However, at pH 4.17 the AlCl_3 sorption level was at 0.72×10^{-4} moles and fell to 0.29×10^{-4} moles at pH 4.30. It is pertinent to note that the ionization range of the carboxyl groups in various representative proteins lies between pII 3 and pH 5 (7).

Interpretation

The data clearly indicate the binding of aluminum by epidermal protein. However, it is difficult to determine the predominant form of aluminum ion which is bound. Although it is likely that Al^{3+} ion and its hydrolysis products, $\text{Al}(\text{OH})^{2+}$ and $\text{Al}(\text{OH})_2^{1+}$ ions, interact with the epidermin, the possibility of some selective process favoring the binding of a specific cation cannot be ruled out. Such a process may depend upon steric factors and electrostatic forces.

If the net charge on the epidermin were negative prior to the binding of aluminum ion, Al^{3+} would be preferentially attracted to the carboxyl binding sites since it has the greatest positive charge. As a result of binding of Al^{3+} , however, the charge on the epidermin would become less negative and could even be reversed into a net positive charge.

Some selectivity in ion binding would also be exerted from the effect of pH upon the epidermin. At a pH below the iso-ionic point the net charge of the epidermin would be more positive. The lower-charge aluminum cations, $\text{Al}(\text{OH})^{2+}$ and $\text{Al}(\text{OH})_2^{1+}$, would be bound in preference to Al^{3+} . On the other hand, if the pH were increased, the negativity of the epidermin would increase and a binding preference for Al^{3+} would be expected. Under the experimental conditions reported here, it is possible that all three of the ionic species of aluminum were sorbed by the epidermin.

From the data in Table I, it may be seen that the binding of aluminum from AlCl_3 passed through a maximum at pH 3.51 and fell off sharply on either side of that pH. This bell-shaped curve clearly suggests the participation of carboxyl groups in the binding process. At lower pH levels the more positively charged epidermin exhibits a decreased avidity for aluminum ions. Furthermore, the high concentration of hydrogen ions keeps the carboxyl groups in the $-\text{COOH}$ form. At higher pH levels, approaching pH 3.51, epidermin loses some of its positive charge, and the carboxyl groups become converted to the $-\text{COO}^-$ state. As a result binding of aluminum increases. At still higher pH levels, above pH 3.51, despite the increasingly favorable state of the epidermin, sorption falls off due to the formation of complex hydrolytic products of aluminum.

A comparison of aluminum sulfate sorption data with those of aluminum chloride (Table I) indicates a considerable difference in the pH-dependence

of binding. Ion-pair formation may be involved in this difference in behavior. Electrostatic attraction between Al^{3+} and SO_4^{2-} should be greater than between Al^{3+} and Cl^- . At low pH's, e. g., 3-4, the larger extent of pairing in the sulfate salt will keep the metal concentration and hence the binding to epidermin lower. However, at somewhat higher pH's, e. g., 4.2, greater ion-pair formation of the sulfate would decrease the extent of hydrolysis of the aluminum ion and hence allow more metal from sulfate than metal from chloride to be bound to epidermin, as is observed in Table I. Ultimately a pII would be reached where even $\text{Al}_2(\text{SO}_4)_3$ would hydrolyze and binding to the epidermin should drop for it too; such a pH was not reached in these studies, but it is obviously above the pH of maximum binding for AlCl_3 .

The maximum amount of aluminum bound by the epidermin from aluminum chloride was 1.66×10^{-4} moles per Gm epidermin (Table II). This is equivalent to 16.6 moles of aluminum per 100,000 Gm epidermin. Assuming that there are approximately 60 free carboxyl groups per 10^5 Gm. epidermin,³ it appears that about three carboxyl groups may interact with each aluminum ion at the pH of maximal sorption.

SUMMARY

Aluminum ion is bound to human keratinous materials in the following *decreasing* order: callus > skin > hair cuticle. Aluminum interaction with (guinea pig) epidermis is strongly dependent upon pH. The ionic species of interacting aluminum may include Al^{3+} , $\text{Al}(\text{OH})^{2+}$, and $\text{Al}(\text{OH})_2^{1+}$. The carboxylate groups of the protein are probably the primary sites of aluminum sorption. Approximately 11 aluminum ions are bound per hydrogen ion released, 3 aluminum ions are bound per chloride ion bound, and 3 chloride ions are bound per hydrogen ion released.

REFERENCES

- (1) Glick, D., "Techniques of Histo- and Cytochemistry," Interscience Publishers, Inc., New York, 1919, p. 21.
- (2) Baumberger, J. P., Surtzoff, V., and Cowdry, E. V., *J. Natl. Cancer Inst.*, **2**, 113 (1912).
- (3) Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," Vol. 1, John Wiley and Sons, Inc., New York, 1947, p. 191.
- (4) Feigl, F., "Chemistry of Specific, Selective, and Sensitive Reactions," Academic Press, Inc., New York, 1919, p. 626.
- (5) Sandell, E. B., "Colorimetric Determination of Traces of Metals," 2nd ed., Interscience Publishers, Inc., New York, 1950, p. 116.
- (6) Laitinen, H. A., Jennings, W. P., and Parks, T. D., *Ind. Eng. Chem.*, **18**, 355 (1916).
- (7) Cohn, E. J., and Edsall, J. T., "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, 1943, p. 358.
- (8) Ward, W. H., and Lundgren, H. P., *Advances in Protein Chem.*, **1X**, 243 (1951).
- (9) Lang, J. M., and Lucas, C. C., *Biochem. J.*, **52**, 84 (1952).
- (10) Block, R. J., *J. Soc. Cosmetic Chemists*, **2**, 235 (1951).
- (11) Block, R. J., and Bolling, D., "The Amino Acid Composition of Proteins and Foods," Charles C. Thomas, Springfield, Ill., 1951, pp. 339, 489.

³ This value was calculated from the aspartic acid, glutamic acid, and amide N data obtained from the analyses of representative keratins (8-11).

Separation of Some Hydroxyanthraquinones by Filter Paper Electrophoresis*

By ALFRED C. CORE† and ERNST R. KIRCH

A method has been developed for the separation of fourteen hydroxyanthraquinones by a hanging strip technique of filter paper electrophoresis. The separation has been applied to chloroform extracts of aloe, cascara, and senna. The method of hydrolysis used converts both anthrone- and anthraquinone-glycosides to hydroxyanthraquinones.

NUMEROUS METHODS may be found in the literature for the qualitative, as well as the quantitative determination of the hydroxyanthraquinone derivatives present in the vegetable laxative drugs. Most of the qualitative methods involve the use of chromatography, either on columns or filter paper strips (1-3), while the quantitative determinations proposed include gravimetric (4), spectrophotometric (1, 2, 4), and biological (5, 6) procedures.

This investigation was undertaken in an effort to accomplish a separation of several hydroxyanthraquinone derivatives by means of electrophoresis on filter paper, a method not previously reported for these compounds.

EXPERIMENTAL

Preparation of Hydroxyanthraquinone Derivatives.—All of the compounds investigated in this study were either obtained commercially or prepared by methods previously reported in the literature, and were purified by recrystallization, followed by sublimation at 0.3 mm. Hg.

Alizarin, anthrarufin, anthraflavic acid, chrysazin, purpurin, and quinalizarin were obtained commercially. Quinizarin was synthesized by the method of Bigelow and Reynolds (7). Chrysophanic acid was prepared from chrysarobin by the procedure of Gardner (8). A portion of the intermediate chrysophanic acid triacetate was oxidized to rhein by the method of Fischer and co-workers (9). Aloe-emodin was obtained by the method of Calm and Simonsen (10). Anthragallol was synthesized by the method of Segui (11). Anthrapurpurin was isolated from a sample of "Alizarin Red-B Paste,"¹ and emodin from the bark of *Rhamnus frangula* by the methods of Hubaehner and co-workers (6). Iso-emodin was isolated from *Cascara sagrada* by the process of Green and co-workers (12).

Preparation of Crude Drug Extracts.—Since the physiological activity of the vegetable laxative drugs, according to Fairbairn (13) and to Stoll and Becker (14), cannot be correlated with free anthraquinone content of the drugs, it seems advisable to disregard such constituents in any chemical estimation of laxative potency.

For this reason, a method of extraction was employed which would remove the free hydroxyanthraquinones before extraction of the glycosides.

One gram of the drug, in fine powder, was extracted in a Soxhlet apparatus with 150 ml. of chloroform, until the solvent remained colorless. This extract, containing the free anthraquinone derivatives, along with other chloroform-soluble coloring materials, fats, and oils, was discarded. The chloroform adhering to the crude drug was removed by drying the apparatus and thimble in air at room temperature. A mixture of 100 ml. of 95% ethanol and 50 ml. of distilled water was then used to extract the drug completely. This extract, containing the glycosides, along with some cleavage products due to partial hydrolysis occurring during this operation, was further treated with hydrochloric acid and hydrogen peroxide in the manner reported by Fischer and Buehegger (4).

Ten milliliters of hydrochloric acid and 2 ml. of 30% hydrogen peroxide were added to the extract and the mixture refluxed on a steam bath for fifteen minutes. The solvent was then evaporated to a volume of 3-5 ml., after which 150 ml. of chloroform was added and the mixture refluxed for thirty minutes. After separation of the chloroform layer, the aqueous portion was extracted with five 50-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and the final volume adjusted with chloroform to 1,000 ml.

Electrophoresis.—The "hanging-strip" method of filter paper electrophoresis described by Williams and co-workers (15) was employed in this investigation.²

Whatman No. 3 mm. filter paper strips, 3 x 30 cm., were used. The compounds were dissolved in approximately 0.1 M aqueous potassium hydroxide, and applied as narrow stripes across the centers of the strips. Solutions of the hydroxyanthraquinones

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¹ Generously supplied by National Aniline Division, Allied Chemical and Dye Corp., New York 6, N. Y.

² The apparatus used was a Spince "Duostat" power supply and Spince Model R, Series B Paper Electrophoresis Cells (Durrum Model).

from the chloroform extracts of the crude drugs were prepared as follows

An aliquot of the chloroform solution was shaken with successive small portions of approximately 0.1 *M* potassium hydroxide solution, until the aqueous layer remained colorless

Electrophoresis was carried out with buffers of various pH values. In some cases, a constant potential was maintained between the electrodes, while in others, a constant current was maintained along the strips

At the completion of the run, the distances to which the compounds had migrated were determined by means of a Beckman Spinco "Analytrol." In cases where the buffer was alkaline, the compounds were present as the highly colored anions of the sodium or potassium salts, and no development of color was necessary. In cases where the buffer was acid, however, it was necessary to develop the colored anions by spraying the strips with 10% ammonium hydroxide solution before analysis. Identification of the compounds was accomplished by running samples of the known compounds on separate strips in the same cell with the mixtures from the crude drug extracts

Of the various combinations of buffers and constant voltages or currents used, a barbital-sodium barbital buffer (0.01 *M* barbital and 0.05 *M* sodium barbital), pH 8.6, ionic strength approximately 0.05, used with a constant applied potential of 400 volts over a period of sixteen hours, gave the best results. The migration of the various hydroxy-anthraquinones under these conditions is shown in Table I

Table II shows the migration of emodin, aloe-emodin and iso emodin with buffers of various pH values

DISCUSSION

As shown in Table I, no two of the compounds displayed exactly the same migration. While some of the values appear to be rather close together, it is possible by the use of the "Analytrol" to identify each compound in a mixture. This is in contrast to the results obtained by Shibata and co workers (3) in paper chromatography of a similar series. They found that of 17 compounds studied, five failed to migrate at all, and many of the remainder had identical *R_f* values, mostly either below 0.05 or above 0.89

Of particular interest is a comparison of the migration distances of emodin, aloe emodin, chrysophanic acid, iso emodin, and rhein with those of the crude drug extracts. It should be noted that no spot

corresponding to chrysophanic acid was observed with any of the drugs examined. While this does not necessarily rule out the possibility of this compound being present, it may suggest that it is not present in sufficient amounts to contribute significantly to the physiological activity. This is in agreement with the findings of Gibson and Schwartz (1) and Brody and co workers (2). The presence of only a single spot on the senna strips is accounted for by the fact that, upon oxidation, the sennosides form only rhein (14) and any other compounds present are in extremely small amounts compared with the sennosides

TABLE I—MIGRATION OF HYDROXYANTHRAQUINONES DURING ELECTROPHORESIS^a

Compound	Substituents	Melting Point, ^b °C	Distance Migrated, mm
Quinizarin	1,4 dihydroxy-	198-199	0 ^c
Alizarin	1,2 dihydroxy-	288-290	2
Purpurin	1,2,4-trihydroxy-	253-255	3
Chrysazin	1,8-dihydroxy-	191-192	4
Anthrarufin	1,5 dihydroxy-	279-280	5
Iso emodin	2,5,8 trihydroxy 5-methyl-	184-186	6
Quinizarin	1,2,5,8-tetrahydroxy-	Above 300	8
Chrysophanic Acid	1,8 dihydroxy-3-methyl-	194-196	9
Anthragalloi	1,2,3-trihydroxy-	306-308	11
Rhein	1,8-dihydroxy-3-carboxylic acid-	309-311	13
Aloe emodin	1,8 dihydroxy-3-hydroxy-methyl-	218-220	14
Anthrapurpurin	1,2,7-trihydroxy-	348-350	15
Emodin	1,6,8-trihydroxy-3-methyl-	257-259	18
Anthraflavic Acid	2,6 dihydroxy-	333-334	52
Aloe Extract			6, 14, 17
Cascara Sagrada Extract			6, 17
Senna Extract			13

^a Barbital-sodium barbital buffer pH 8.6 ionic strength 0.05 constant potential 400 v sixteen hours duration

^b All melting points uncorrected

^c Quinizarin failed to migrate in any of the experiments conducted

TABLE II—MIGRATION OF EMODIN, ALOE-EMODIN AND ISO EMODIN IN BUFFERS OF VARIOUS pH VALUES

pH	2	4.0	8.6 ^a	8.6 ^b	9.2 ^c	9.2 ^d
Emodin	6 mm	1 mm	18 mm	17 mm	17 mm	7 mm
Aloe emodin	7 mm	2 mm	14 mm	13 mm	11 mm	14 mm
Iso emodin	6 mm	0 mm	6 mm	8 mm	6 mm	4 mm

^a Barbital sodium barbital ionic strength 0.05, sixteen hours

^b Barbital sodium barbital ionic strength 0.08, twenty hours

^c Sodium borate 0.01 *M*

^d Barbital sodium barbital ionic strength 0.1

While the separation of the compounds was always reproducible, the migration could not be shown to bear a linear relationship to time with the apparatus used. This is in accordance with the findings of other investigators in the field using similar apparatus (16, 17), and is explained by the lack of a uniform ratio of water to paper throughout the strip.

A similar lack of linearity was encountered with the use of various potentials, and can be attributed to the same factor.

Results were reproducible when a constant potential was maintained, but the migration was found to be erratic when a constant current was passed through the strips. This is in agreement with the findings of Raymond (18). Since the migration of a charged particle in an electric field depends upon the field strength in volts per centimeter length of the paper strip between buffer surfaces, changes in the resistance along the strip will create a constantly varying potential in order to maintain a constant current.

There does not seem to be a correlation between either the number of functional groups or their position on the molecule, and rate of migration. A possible explanation of this behavior may lie in the fact that all of the hydroxyl groups do not necessarily form salts at the pH of the buffer used.

By the use of the extraction method reported here, the quantitative determinations are based only on glycosidal content. Other investigators have determined total anthraquinones content (1, 2, 19), and discarded the anthrones. In the case of cascara, Fairbairn has demonstrated (13) that free hydroxyanthraquinones contribute little if anything to the laxative potency of the drugs, while the anthrone glycosides do. Furthermore, Stoll and Becker (14) have found the chief active constituents of senna to be dianthrone-glycosides which are not readily converted to anthraquinone derivatives by acid hydrolysis alone.

It seems advisable, therefore, that in any chemical estimation of the laxative potency of these drugs, the free hydroxyanthraquinone content be disregarded, and the anthrone-glycoside content, together with hydroxyanthraquinone-glycoside content, be used as the basis for assay. Since the anthrones are readily oxidized to anthraquinones by hydrogen peroxide, extracts of the drugs may be prepared containing only hydroxyanthraquinones, but at the same time representing the total glycoside content. This is accomplished by adding the hydro-

gen peroxide to the reaction mixture during hydrolysis of the glycosides. Removal of free anthraquinones may be achieved by a preliminary extraction of the drug with chloroform.

A method of quantitative estimation, based on the fluorescence of the hydroxyanthraquinones found in the vegetable laxatives, is under study.

SUMMARY

1. Separation of fourteen different hydroxyanthraquinones has been accomplished by the hanging-strip technique of filter paper electrophoresis. While no linear relationship has been shown to exist between time and migration, the compounds have consistently migrated to the same relative positions on the paper.

2. A method for preparing extracts of the vegetable laxative drugs containing these compounds for use in quantitative determinations is proposed. This method eliminates the free hydroxyanthraquinone content, but converts the entire glycoside content to anthraquinones.

REFERENCES

- (1) Gibson, M. R., and Schwarting, A. E., *THIS JOURNAL*, **37**, 206(1948).
- (2) Brody, T. M., Voigt, R. F., and Maher, F. T., *ibid.*, **39**, 666(1950).
- (3) Shibata, S., Takito, M., and Tanaka, O., *J. Am. Chem. Soc.*, **72**, 2789(1950).
- (4) Fischer, R., and Buchegger, E., *Pharm. Zentralhalle*, **89**, 261(1950).
- (5) Lou, T. C., *J. Pharm. and Pharmacol.*, **1**, 673(1949).
- (6) Hubacher, M. H., Doernberg, S., and Horner, A., *THIS JOURNAL*, **42**, 23(1953).
- (7) Bigelow, L. A., and Reynolds, H. H., "Organic Syntheses, Coll. Vol. I," John Wiley and Sons, Inc., New York, 1944, pp. 476-478.
- (8) Gardner, J. H., *THIS JOURNAL*, **23**, 1178(1934).
- (9) Fischer, O., Flaco, F., and Gross, H., *J. prakt. Chem.*, **83**, 208(1911).
- (10) Cahn, R. S., and Simonsen, J. L., *J. Chem. Soc.*, **135**, 2573(1932).
- (11) Segui, L., *Quim. e ind. (Barcelona)*, **11**, 81(1934).
- (12) Green, M. W., King, C. G., and Beal, G. D., *THIS JOURNAL*, **27**, 95(1938).
- (13) Fairbairn, J. W., *J. Pharm. and Pharmacol.*, **1**, 683(1949).
- (14) Stoll, A., and Becker, B., "Fortschritte der Chemie organischer Naturstoffe," VII, Vienna, 1950, pp. 248-69.
- (15) Williams, F. G., Jr., Pickels, E. G., and Durrum, E. L., *Science*, **121**, 829(1955).
- (16) Durrum, E. L., *J. Am. Chem. Soc.*, **72**, 2943(1950).
- (17) McDonald, H. J., *J. Chem. Educ.*, **29**, 428(1952).
- (18) Raymond, S., *Science*, **120**, 677(1954).
- (19) Mary, N. Y., Christensen, B. V., and Beal, J. T., *THIS JOURNAL*, **45**, 229(1956).

Excretion and Distribution Studies with *o*-, *m*-, and *p*-Chloroacetanilide-Cl³⁶ in Rats and Guinea Pigs*

By MARY F. ARGUS, JOSEPHINE M. GRYDER, TREVA L. SEEPE,
GEORGE CALDES, and FRANCIS E. RAY†

o-, *m*- and *p*-Chloroacetanilide-Cl³⁶ in methylcellulose suspensions were injected intraperitoneally into rats and guinea pigs. The excretion pattern of each isomer was determined and distribution studies of the three compounds in both species at various times after administration were compared.

ACETANILIDE has proved of value to man as an antipyretic and analgesic drug. The metabolism of acetanilide involves deacetylation and hydroxylation of the ring, with both *p*- and *o*-hydroxyaniline being formed (1). *In vitro* experiments with rat liver extracts have shown that ring substitution affects the extent of deacetylation (2). Such substitution may also affect the position and extent of hydroxylation of acetanilide. This in turn would possibly alter the toxicity and physiological properties of the compound. Accordingly *o*-, *m*-, and *p*-chloroacetanilide-Cl³⁶ were prepared (3, 4), and an excretion and distribution study with the *p*-isomer was first carried out using propylene glycol as an injection medium (3). It was later pointed out that this solvent is toxic to the experimental animals employed and changes the blood picture in animals injected with *p*-chloroacetanilide (5). Rats and guinea pigs have been found to respond differently in their metabolism (6, 7) and detoxication (8), and in their response to the carcinogenic action of such compounds (9). Species differences were also found in the excretion and distribution of *p*-chloroacetanilide in the previous study with propylene glycol solvent (3).

The present study was undertaken to determine the effect of the position of chlorine-ring-substitution in acetanilide on excretion and distribution patterns. Both rats and guinea pigs were studied and each Cl³⁶-compound was administered in methylcellulose suspension which is nontoxic at the levels used.

EXPERIMENTAL

The methods are the same as those previously

reported (3) except for the following details. Fifteen male stock guinea pigs (average weight 666 Gm.) and twelve male Sprague-Dawley rats (average weight 338 Gm.) were employed. The chloroacetanilide-Cl³⁶-isomers were suspended in 2% aqueous methylcellulose (Methocel) in a concentration of 25 mg. compound/ml. suspension. The specific activities of the injected compounds were: *o*-chloroacetanilide-Cl³⁶, 70,452 d/m/mg.; *m*-chloroacetanilide-Cl³⁶, 138,160 d/m/mg.; *p*-chloroacetanilide-Cl³⁶, 85,837 d/m/mg. Excreta from one rat and one guinea pig injected with each compound was collected at one to twelve hour intervals over at least one hundred and twenty hours or until no activity was detectable in the urine and feces. Distribution studies were carried out at the time of maximum excretion. Some studies were also made at intervals less than and greater than the peak excretion times.

Water washings from the abdominal cavity and organs were designated "abdominal fluid." Residual urine was added to the excreted urine. In some cases the gall bladder fluid of the guinea pigs was separately examined. The feces and contents of the gastrointestinal tract were dried at room temperature for forty-eight hours. Following removal of the organs, the bones were removed from the carcass and soaked for forty-eight hours in 10% HCl. The carcass (skin, muscles, and subcutaneous tissue) was minced well with scissors and soaked for twenty-four hours in 20% NaOH to facilitate disintegration. Prior to analysis the concentration was reduced to that employed for the other organs.

A 1-ml. portion of each sample was then placed on a stainless steel planchet and was dried at room temperature. The bones were minced with scissors, and enough 20% NaOH was added to the acid solution to bring the volume to the same proportion used for the organs and carcass. The mixture was homogenized and a 1-ml. portion plated as before. One-milliliter samples of urine, blood cells, blood plasma, abdominal fluid, and gall bladder fluid were plated directly on planchets.

Urine standards were used for the urine, blood plasma, abdominal fluid, and gall bladder fluid. Total doses recovered from the blood were calculated from blood volumes based on the average of 6.7 ml. of blood per 100 Gm. of body weight (10).

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† The authors gratefully acknowledge the assistance of Miss Marjorie Newell in the preparation of the Cl³⁶-labeled compounds employed in this study.

RESULTS AND DISCUSSION

Fecal elimination is the secondary route of removal of the monochloro, ring-substituted acetanilides: the largest per cent of the administered dose is recovered from the urine. For each compound the per cent eliminated in the feces is greater for the rat than for the guinea pig (Table I). Peak excretion of *o*-chloroacetanilide in the urine occurs at six hours in the rat and at three hours in the guinea pig. Elimination from both species is complete at eighty-four hours (Fig. 1). The *m*-compound shows the same times of peak excretion, but traces of activity continue to be excreted five hundred hours after intraperitoneal administration (Fig. 2). The *p*-derivative does not reach a peak excretion in the rat and guinea pig until twenty-four and thirty-six hours (Fig. 3). Traces of activity are present in the urine at one hundred and twenty hours with this compound. Of the three, the *o*-chloro isomer is thus seen to be the most readily eliminated from the animal body.

Metabolism of acetanilide occurs in the rodent principally by oxidation to *p*-hydroxyaniline (1). It is not surprising, therefore, that *p*-chloroacetanilide, in which the important *p*-position is blocked, reaches peak excretion later in the urine than the *o*- and *m*-chloro isomers. *p*-Substitution also influences the toxicity of the compound. Recent LD₅₀ studies with ring-substituted, chloroacetanilide isomers in Sprague-Dawley rats gave the order of decreasing toxicity: *p*-, *m*-, *o*- (11).

The time required for peak excretion of the *p*-compound by the guinea pig is 12 times that required for the *o*- and *m*-compounds, while in the rat the *p*-isomer peak excretion time is only four times that needed for the *o*- and *m*-compounds. This may reflect differences in the hydroxylation mechanisms of the two species. Making use of this line of reasoning Weisburger, *et al.* (8), were able to demonstrate such a difference. They showed the presence of *o*-hydroxylated derivatives of 2-acetylaminofluorene in the urine of the rat but failed to find these derivatives in the urine of the guinea pig. These facts were used to explain the resistance of the guinea pig

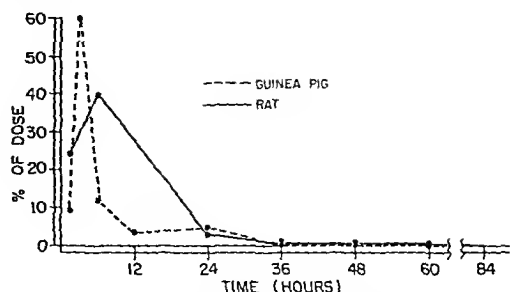


Fig. 1.—Urinary excretion of 100 mg. *ortho*-chloroacetanilide-Cl³⁶/Kg. by the rat and the guinea pig.

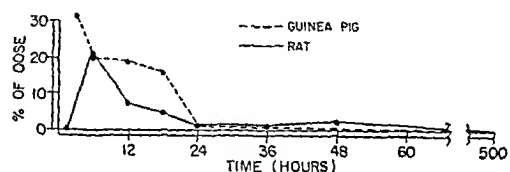


Fig. 2.—Urinary excretion of 100 mg. *meta*-chloroacetanilide-Cl³⁶/Kg. by the rat and the guinea pig.

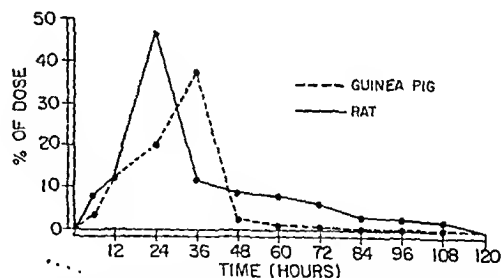


Fig. 3.—Urinary excretion of 100 mg. *para*-chloroacetanilide-Cl³⁶/Kg. by the rat and the guinea pig.

TABLE I.—PER CENT OF ADMINISTERED DOSE APPEARING IN URINE AND FECES OF RATS AND GUINEA PIGS FOLLOWING INTRAPERITONEAL INJECTIONS OF *o*-, *m*- AND *p*-CHLOROACETANILIDE

Hours After Administration	Isomer	Species	% of Dose	
			Urine	Feces
84	<i>Ortho</i>	Rat	87.22	0.68
84	<i>Ortho</i>	Guinea Pig	90.80	0.36
500	<i>Meta</i>	Rat	40.56 ^a	2.10
500	<i>Meta</i>	Guinea Pig	97.16	1.06
120	<i>Para</i>	Rat	92.77	5.56
120	<i>Para</i>	Guinea Pig	87.83	1.74

^a In 5 rats the total recovery varied from 40.56% to 87.00%. The time of peak excretion remained the same.

and the susceptibility of the rat to the carcinogenic activity of this compound (9).

When *p*-chloroacetanilide was administered in propylene glycol, elimination was then more rapid in the guinea pig than in the rat. Peak excretion occurred for the guinea pig at twelve hours (75.7%) and for the rat at twenty-four hours (68.9%). The rat required eighty-four hours to eliminate completely the administered dose while the guinea pig required seventy-two hours (3). By comparison with the data obtained when this compound is

injected in methylcellulose suspension (Fig 3), it is seen that the period required for total excretion is longer with the latter medium. For both species the per cent of administered dose recovered at the time of peak excretion is substantially less than that eliminated when propylene glycol is the solvent. The effect due to the injection medium is more pronounced for the guinea pig than for the rat.

The distribution of the three chloroacetanilides in the animal body at various times after injection in methylcellulose suspension is summarized in Tables II, III, and IV. The concentration of compound in the blood plasma exceeds that in blood cells in all cases except for the *p*-compound in the rat. This species difference in the handling of the most toxic isomer is also evident in the relative concentrations of radioactivity in the liver and kidneys. The guinea pig shows a higher level of *p*-compound in the kidneys than in the liver, while the rat localizes more of the *p*-isomer in the liver than in the kidneys. This retention by the kidneys of the guinea pig is reflected in the later peak excretion time for this species. On the other hand, the more rapidly excreted compounds (*o*- and *m*-) are present in greater concentrations in the kidneys than in the liver of both species at all of the times studied. The mechanisms for these compounds appear to be different. In the case of the *p*-compound the kidneys seem to be the limiting factor in the guinea pig, while in the rat the rate of metabolism by the liver is probably the limiting factor. For the *o*- and *m*-compounds the kidneys appear to be the controlling factor.

Although the compounds were injected into the peritoneal cavity, substantial amounts are found in

TABLE III.—DISTRIBUTION OF RADIOACTIVITY FROM *m*-CHLOROACETANILIDE-CL³⁶ IN THE RAT AND GUINEA PIG 3.5 AND 6 HOURS AFTER INTRAPERITONEAL INJECTION OF 100 MG /KG

Tissue	Concn μ g/Gm Tissue ^a		% of Dose—Guinea Pig	
	Rat, 6 Hr	Guinea Pig, 3.5 Hr	Rat, 6 Hr	Guinea Pig, 3.5 Hr
Blood cells	35 00	0 00	1 00	0 00
Blood plasma	74 00	33 00	2 91	1 20
Liver	60 00	13 00	2 20	0 45
Spleen	30 00	28 00	0 03	0 02
Kidneys	130 00	97 00	0 94	0 67
Stomach	60 00	11 00	0 26	0 07
Small intestine	180 00	160 00	2 80	2 00
Cecum	47 00	58 00	0 14	0 48
Colon	53 00	55 00	0 15	0 38
Stomach contents	160 00	68 00	0 55	0 44
Small intestine contents	260 00	1,500 00	0 26	2 50
Cecum contents	230 00	330 00	0 55	4 50
Colon contents	30 00	85 00	0 05	0 38
Miscellaneous	0 70	23 00	0 04	0 93
Abdominal fluid	64 00	177 50	0 83	1 70
Bones	48 00	17 00	10 60	3 40
Carcass	70 00	22 00	36 90	9 90

^a Or μ g /ml blood cells or blood plasma

the gastrointestinal tract. Largest recoveries are from the intestinal tract of both species at the shortest time intervals.

TABLE II.—DISTRIBUTION OF RADIOACTIVITY FROM *o*-CHLOROACETANILIDE-CL³⁶ IN THE RAT AND GUINEA PIG 3, 6, AND 12 HOURS AFTER INTRAPERITONEAL INJECTION OF 100 MG /KG

Tissue	Concn μ g/Gm Tissue ^a						% of Dose—Guinea Pig				
	Rat			Guinea Pig			Rat			Guinea Pig	
	3 Hr ^b	6 Hr ^b	12 Hr ^b	3 Hr ^b	6 Hr ^b	12 Hr ^b	3 Hr ^b	6 Hr ^b	12 Hr ^b	3 Hr ^b	6 Hr ^b
Blood cells	2 13	0 65	0 80	0 35	0 60	0 07	0 03	0 11	0 02	0 02	0 02
Blood plasma	4 65	5 90	7 80	5 00	3 40	0 16	0 22	0 27	0 17	0 11	0 11
Liver	5 00	1 50	4 13	7 50	2 75	0 21	0 07	0 21	0 30	0 11	0 11
Spleen	0 63	0 63	1 50	0 63	0 75	<0 01	<0 01	<0 01	<0 01	<0 01	<0 01
Kidneys	13 50	4 13	18 38	13 00	8 75	0 11	0 04	0 23	0 12	0 08	0 08
Stomach	1 00	0 63	4 38	1 63	1 50	<0 01	<0 01	0 04	0 01	0 01	0 01
Small intestine	66 48	10 63	19 88	5 25	1 75	1 16	0 26	0 38	0 14	0 02	0 02
Cecum	2 13	4 63	3 38	6 13	3 50	0 01	0 02	0 07	0 09	0 04	0 04
Colon	2 48	3 63	2 00	1 63	0 75	0 01	0 02	0 03	0 02	<0 01	<0 01
Stomach contents	9 75	11 00	132 50	109 75	69 00	0 03	0 04	0 24	0 22	0 06	0 06
Small intestine contents	406 50	283 75	460 75	767 50	47 00	3 40	0 79	1 37	0 47	0 04	0 04
Cecum contents	9 50	95 00	6 00	61 00	10 00	0 03	0 27	0 04	0 48	0 02	0 02
Colon contents	4 25	134 25	6 75	10 50	76 50	<0 01	0 05	0 04	0 05	0 44	0 44
Miscellaneous	2 23	1 50	2 25	5 13	2 50	0 07	0 06	0 09	0 25	0 12	0 12
Abdominal fluid	126 50	1 00	1 00	2 35	0 50	2 16	0 12	0 15	0 36	0 01	0 01
Bones	0 88	0 63	0 63	0 50		0 18	0 14	0 12	0 08		
Carcass	1 00	1 13	0 75	1 38	0 50	0 72	0 70	0 55	0 85	0 38	0 38

^a Or μ g /ml. blood cells or blood plasma

^b Average value from 2 animals

TABLE IV — DISTRIBUTION OF RADIOACTIVITY FROM *p*-CHLOROACETANILIDE- Cl^{36} IN THE RAT AND GUINEA PIG 6, 18, 24, AND 36 HOURS AFTER INTRAPERITONEAL INJECTION OF 100 MG./KG.

Tissue	Concn $\mu\text{g}/\text{Gm}$		Tissues ^a			% of Dose				
	Rat		Guinea Pig			Rat		Guinea Pig		
	6 Hr ^b	24 Hr ^b	6 Hr ^b	18 Hr ^b	36 Hr ^b	6 Hr ^b	24 Hr ^b	6 Hr ^b	18 Hr ^b	36 Hr ^b
Blood cells	90 50	245 60	3 75	7 75	0 75	3 14	8 02	0 15	0 31	0 03
Blood plasma	27 75	30 00	43 95	34 40	5 80	0 96	0 99	1 18	3 22	0 16
Liver	98 15	97 50	75 00	74 50	9 00	3 80	4 20	2 86	2 49	0 33
Spleen	32 50	57 50	58 00	52 00	5 95	0 07	0 06	0 06	0 06	0 01
Kidneys	59 90	76 90	94 50	108 00	12 50	0 47	0 48	0 81	0 86	0 11
Stomach	87 50	63 75	42 75	51 50	8 75	0 45	0 33	0 42	0 38	0 07
Small intestine	145 00	160 65	50 00	59 00	7 45	2 52	2 13	0 22	0 96	0 12
Cecum	75 65	54 65	35 25	53 00	9 50	0 24	0 10	0 60	0 53	0 11
Colon	87 50	44 90	35 25	46 50	4 00	0 50	0 19	0 38	0 52	0 03
Stomach contents	55 25	60 00	100 50	598 00	88 00	0 11	0 61	0 02	0 31	0 05
Small intestine contents	692 50	1332 50	406 00	512 00	74 50	0 98	3 09	0 45	0 31	0 06
Cecum contents	112 50	351 00	153 00	313 50	61 00	0 30	0 45	1 60	2 27	0 50
Colon contents	92 25	333 00	59 00	89 50	57 25	0 16	0 40	0 28	0 03	0 07
Miscellaneous	106 25	58 15	84 00	101 00	7 50	5 92	4 35	2 96	4 10	0 28
Abdominal fluid	4 65	3 25	4 30	17 50	1 25	1 62	1 77	0 02	0 03	<0 01
Bones	30 25	30 00	36 00	44 75	6 50	5 95	7 41	7 92	5 29	1 89
Carcass	72 80	16 50	157 15	166 35	15 05	49 34	10 02	74 44	53 39	4 14
Gall bladder fluid			64 00	43 00	17 70			0 15	0 05	0 04

^a Or $\mu\text{g}/\text{ml}$ blood cells or blood plasma^b Average value from 2 animals

The per cent of *p*-chloroacetanilide recovered from the carcass, bones, and miscellaneous organs is in general greater than that found for the *o*- and *m*-compounds. It appears that the *p*-isomer is stored to the largest extent before elimination, probably because chlorosubstitution in the *p*-position interferes with the rate of hydroxylation and conversion to the more soluble glucuronides and ethereal sulfates.

SUMMARY

1. The excretion and distribution patterns of *o*-, *m*-, and *p*-chloroacetanilide- Cl^{36} were determined in rats and guinea pigs following intraperitoneal injection.

2. The *o*- and *m*-isomers reached peak excretion more readily in the guinea pig, while *p*-chloroacetanilide reached a peak excretion in the guinea pig more slowly than in the rat.

3. Species differences were observed in the distribution of the *p*-compound in the blood, liver, and kidneys.

4. With all three isomers substantial amounts of radioactivity were found in the gastrointestinal tract of both species.

5. *p*-Chloroacetanilide was retained by the carcass, bones, and miscellaneous organs to a greater extent by both species than were the *o*- and *m*-compounds.

REFERENCES

- (1) Wilhams, R. T., "Detoxication Mechanisms," John Wiley and Sons, Inc., New York, 1947, p. 143
- (2) Bray, H. G., James, S. P., Thorpe, W. V., and Wasdell, M. R., *Biochem J.*, **47**, 483(1950)
- (3) Gryder, J. M., Argus, M. F., Newell, M. P., and Ray, F. E., *This Journal*, **43**, 667(1954)
- (4) Newell, M. P., Argus, M. F., and Ray, F. E., *J. Am. Chem. Soc.*, **78**, 6122(1956)
- (5) Glocklin, V. C., *Proc. Soc. Exptl. Biol. Med.*, **85**, 663(1954)
- (6) Gutmann, H. R., Kiley, G. E., and Klein, M., *Cancer Research*, **12**, 350(1952)
- (7) Meade, J. M., and Ray, F. E., *Arch. Biochem. and Biophys.*, **49**, 43(1954)
- (8) Veisburger, E. K., and Morris, H., and Argus, M. F., *Quart. J. Florida Acad. Sci.*, **19**, 68(1956)
- (9) Farris, E. J., and Griffith, J. D., "The Rat in Laboratory Investigation," J. B. Lippincott Company, Philadelphia, 1949, p. 413
- (10) Argus, M. F. and Newell, M. P., In press

Spectrophotometric Determination of Steroid Esters*

By ARLINGTON A. FORIST and SUSAN THEAL

Modifications of the hydroxamic acid procedure for esters have been developed and applied to the determination of a variety of steroid esters. The methods presented are rapid, accurate, and precise (mean recovery = standard deviation 99.9 ± 1.4%).

MANY STEROIDS possess superior properties such as more efficient absorption or prolonged duration of action when administered in the form of various esters. However, the usual assay procedures for such drugs do not include a determination of the ester content. In view of the significance of such a measurement, the general procedure of Goddu, LeBlanc, and Wright (1) has been modified and applied to the determination of a variety of steroid esters as described herein.

EXPERIMENTAL

Reagents—(a) Hydroxylamine hydrochloride, 12.5%, in methanol. This solution is about 1.8 *M*.

(b) Sodium hydroxide, 12.5%, reagent grade, in 85% methanol. A sample of 12.5 Gm. of sodium hydroxide is dissolved in 15 cc. of water and, on cooling, the resulting solution is diluted to 100 cc. with methanol. This solution is about 3.1 *M*.

(c) Ferric perchlorate, stock solution. An 800 mg. portion of iron powder is mixed with 3 cc. of water in a 50 cc. beaker. Ten cubic centimeters of 70% perchloric acid and 7 cc. of water are then slowly added dropwise. If small particles of iron are not dissolved, the process may be hastened by gentle heating or the sample may be set aside overnight to allow complete solution. The resulting solution is quantitatively transferred to a 100 cc. volumetric flask with anhydrous 2B ethanol and, with cooling under running tap water, gradually diluted to 100 cc. with anhydrous 2B ethanol.

(d) Ferric perchlorate, reagent solution. A 40 cc. aliquot of the stock solution is transferred to a 1-L. volumetric flask followed by 12 cc. of 70% perchloric acid. The resulting solution is gradually diluted to 1 L. with anhydrous 2B ethanol with cooling under running tap water. The ferric ion concentration of this solution is 0.0057 *M* and the perchloric acid concentration is 0.16 *M*.

(e) Alkaline hydroxylamine reagent. Equal volumes of the 12.5% hydroxylamine hydrochloride and 12.5% sodium hydroxide solutions are mixed

and the insoluble sodium chloride is removed by filtration through Whatman No. 40 paper. This reagent solution should be prepared immediately before use and is usable for four hours.

(f) Standard steroid ester solution. An approximately 0.008 *M* solution of a standard sample of the steroid ester to be determined is prepared by dissolving an accurately weighed sample in anhydrous 2B ethanol and accurately diluting the resulting solution to an appropriate volume with anhydrous 2B ethanol.

Apparatus.—A Beckman Model B spectrophotometer with 1-cm. cells has been employed for absorbance measurements. A 50° constant temperature water bath is required for Procedure B.

Procedure A.—An approximately 0.005 *M* solution of the steroid ester to be determined is prepared by transferring an accurately weighed sample (45–60 mg. depending on the steroid ester involved) to a 25 cc. volumetric flask, dissolving in anhydrous 2B ethanol, and diluting the resulting solution to 25 cc.

A 5 cc. aliquot of the sample solution is transferred to a 50 cc. volumetric flask followed by 3 cc. of the alkaline hydroxylamine reagent. The solution is mixed and reaction allowed to proceed at room temperature for from ten to sixty minutes depending on the nature of the steroid ester being determined. At the end of that time, the solution is diluted to about 40 cc. with the ferric perchlorate reagent, thoroughly mixed, and stored in the absence of light for about ten minutes. (Usually several samples are run simultaneously and each is brought to this stage before proceeding further.) The solution is then diluted to 50 cc. with the ferric perchlorate reagent and thoroughly mixed. The absorbance of the resulting solution is measured within one hour at 530 mμ in a 1 cm. cell *versus* a reagent blank similarly prepared. The sample should be stored in the dark between the final dilution and the absorbance determination.

Two standard samples are run in parallel with the unknown. Five cubic centimeters of the standard ester solution are added to one 50 cc. volumetric flask, 2 cc. of the standard solution and 3 cc. of anhydrous 2B ethanol are added to a second 50 cc. volumetric flask. Each sample is carried through the procedure described above.

The per cent of the desired steroid ester in the sample is calculated from the equation

$$\% \text{ Ester} = \frac{A_{530} \times 100}{a_{530} \times C}$$

where

A_{530} = observed absorbance at 530 mμ for the sample

a_{530} = absorbance per mg. per cc. for the standard at 530 mμ

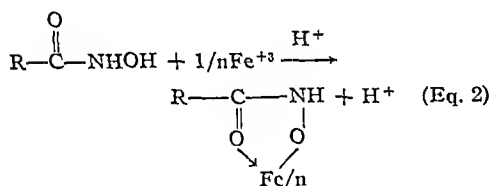
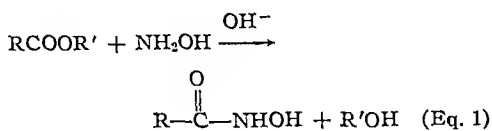
C = mg. of sample per cc. of solution

* Received October 3, 1957, from the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

Procedure B.—A 5-cc. aliquot of the sample solution, prepared as in Procedure A, is transferred to a 50-cc. volumetric flask followed by 3 cc. of the alkaline hydroxylamine reagent. The solution is mixed and the tightly-stoppered flask is immersed in a 50° constant temperature water bath for one hour. The flask is then removed and shaken under running tap water while the solution is diluted to about 40 cc. with the ferric perchlorate reagent. The remainder of the procedure is the same as Procedure A, above.

RESULTS AND DISCUSSION

Various methods have been described for the determination of esters based on the production of hydroxamic acids (Eq. 1) followed by conversion of the hydroxamic acids to their highly colored ferric complexes (Eq. 2) (2-8).



Goddu, LeBlanc, and Wright (1) have recently made a thorough study of this reaction and have described an excellent general procedure for esters. However, reaction under reflux was required in order to measure the esters of aromatic and fatty acids. Since the present needs are more specific than general, less vigorous conditions have been sought which would yield a method more easily adaptable to the analysis of large numbers of samples.

Formation of hydroxamic acids from a group of steroid esters at room temperature is shown in Table I. Reaction with hydrocortisone acetate (FAc) and prednisolone acetate (Δ^1 -FAc) is complete within five minutes. In the case of hydrocortisone cyclopentylpropionate (FCP) and prednisolone cyclopentylpropionate (Δ^1 -FCP), twenty to thirty minutes are required for complete reaction whereas 17 α -acetoxyprogesterone (17-AcPr) gives a maximum yield of hydroxamic acid after forty minutes. In all cases, the hydroxamic acid is stable in the reaction mixture. Figure 1 shows a similar study with testosterone cyclopentylpropionate (TCP). With this ester, reaction at room temperature is incomplete even after one hundred minutes, but at 50°, hydroxamic acid production is maximal within fifty minutes. In routine use, Procedure A is applied to steroid esters such as those shown in Table I where reaction is complete within one hour at room temperature. Other steroid esters, such as TCP, are determined by Procedure B. Conditions required for maximal production of

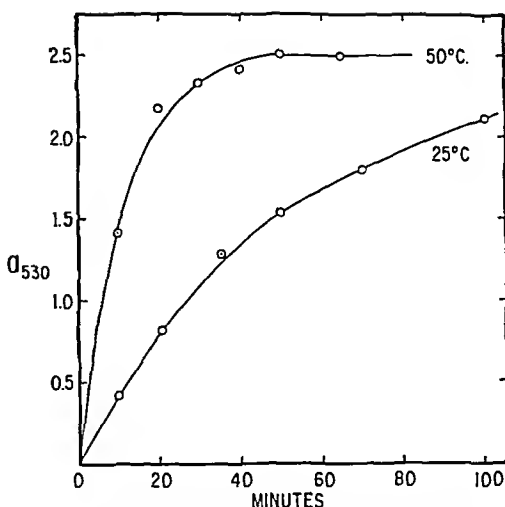


Fig. 1.—Production of hydroxamic acid from testosterone cyclopentylpropionate.

hydroxamic acids from the various steroid esters studied appear to reflect steric factors.

The various ferric hydroxamates examined have shown absorption maxima at 530 mμ. Results in Table II show that ferric acetoxyhydroxamate is stable under the conditions employed for at least one hour in the absence of light. Similar results have been obtained with ferric cyclopentylpropionohydroxamate. The ferric hydroxamates are photosensitive, however, and should not be exposed to direct light.

TABLE I.—PRODUCTION OF HYDROXAMIC ACIDS FROM STEROID ESTERS AT ROOM TEMPERATURE

Time, min.	Absorptivity at 530 mμ				
	FAc	Δ^1 -FAc	FCP	Δ^1 -FCP	17-AcPr
5	2.84	2.89
10	2.84	2.96	2.22	1.92	2.09
20	2.83	2.94	2.30	2.18	2.28
30	2.82	2.90	2.32	2.21	2.33
40	2.32	2.21	2.40
50	2.34	2.22	2.39
60	2.80	...	2.34	2.21	2.37

TABLE II.—STABILITY OF FERRIC ACETOXYHYDROXAMATE IN THE ABSENCE OF LIGHT

Time After Addition of Fe(ClO ₄) ₃ , min.	Absorptivity at 530 mμ ^a
10	2.34
20	2.37
30	2.37
40	2.36
50	2.37
60	2.38

^a From 17-AcPr.

Beer's law is obeyed for steroid ester concentrations of 0-0.01 *M* in the sample solution (A_{630} 0-1.0). The response varies somewhat from day to day, however, and for maximum accuracy standards should be run in parallel with unknown samples as described above.

Representative data obtained in the application of Procedures A and B to standard steroid esters are presented in Tables III and IV, respectively, and indicate excellent accuracy and precision. Typical results obtained in the analysis of a group of 17 α -acetoxyprogesterone samples by each of two operators are shown in Table V. Agreement is excellent.

The methods presented are rapid, accurate, and precise. Hydrocortisone gives less than 1% of the response obtained with its esters indicating no interference due to the presence of nonesterified steroid. Other functional groups capable of yielding hydroxamic acids under the conditions employed (such as anhydrides, acid chlorides, lactones, and imides) constitute positive interferences. High concentrations of carbonyls, transition elements,

TABLE IV—DETERMINATION OF STANDARD TESTOSTERONE CYCLOPENTYLPROPIONATE—PROCEDURE B

Taken, mg /cc	Found, mg /cc	Recovery, %
3.23	3.28	101.5
2.42	2.43	100.4
2.02	2.03	100.5
1.62	1.61	99.4
1.21	1.21	100.0
0.81	0.79	97.5
0.40	0.40	100.0
Mean \pm standard deviation 99.9 \pm 1.2		

TABLE V.—ANALYSIS OF 17 α -ACETOXYPROGESTERONE SAMPLES—PROCEDURE A

Sample	% 17-AcPr	
	Operator A	Operator B
1	98.5	97.7
2	100.0	99.4
3	100.9	99.4
4	100.3	99.7
5	97.3	96.3

TABLE III—DETERMINATION OF STANDARD STEROID ESTERS—PROCEDURE A

Steroid Ester	Taken mg /cc	Found mg /cc	Recovery %
FAc	2.63	2.63	100.0
	1.97	1.99	101.0
	1.31	1.35	103.1
	0.66	0.67	101.5
Δ^1 -FAc	3.33	3.34	100.3
	2.66	2.68	100.8
	2.00	2.00	100.0
	1.33	1.33	100.0
	0.67	0.67	100.0
FCP	3.41	3.45	101.2
	2.73	2.73	100.0
	2.05	2.03	99.0
	1.37	1.35	98.5
	0.68	0.66	97.1
Δ^1 -FCP	3.21	3.23	100.6
	2.57	2.56	99.6
	1.93	1.90	98.4
	1.28	1.29	100.8
	0.64	0.62	96.9
17-AcPr	4.09	4.11	100.5
	3.27	3.30	100.9
	2.45	2.42	98.8
	1.64	1.60	97.6
	0.82	0.82	100.0
Mean \pm standard deviation 99.9 \pm 1.4			

and ions capable of forming complexes with ferric iron will affect the intensity of the color and should be avoided.

SUMMARY

Modified hydroxamic acid procedures have been developed and applied to the determination of a variety of steroid esters. The methods presented are rapid, accurate, and precise.

REFERENCES

- (1) Goddu, R. F., LeBlanc, N. F., and Wright, C. M., *Anal. Chem.*, **27**, 1251 (1955).
- (2) Hill, U. T., *Ind. Eng. Chem., Anal. Ed.*, **18**, 317 (1946).
- (3) Hill, U. T., *Anal. Chem.*, **19**, 932 (1947).
- (4) Bauer, F. C., Jr., and Hirsch, E. F., *Arch. Biochem.*, **20**, 242 (1949).
- (5) Hestrin, S., *J. Biol. Chem.*, **180**, 249 (1949).
- (6) Buckels, R. E., and Thelen, C. J., *Anal. Chem.*, **22**, 676 (1950).
- (7) Zaffaroni, A., *Recent Prog. in Hormone Research*, **8**, 51 (1953).
- (8) Naylor, R., Bauer, F. C., Jr., and Hirsch, E. F., *Arch. Biochem. and Biophys.*, **54**, 201 (1955).

The Use of Microscopic and X-ray Diffraction Methods for the Identification of Sedatives and Anticonvulsants*

By W. G. PENPRASE† and JOHN A. BILES

Crystals of the newer barbiturates, the amides, and imides used as sedatives and anticonvulsants, were obtained by fusion, sublimation, crystallization from 50% alcohol, and crystallization from concentrated ammonia. Photographs of the different crystalline habits illustrate this method to be a useful means of identification. X-ray diffraction powder diagrams were made and the d-distances are reported.

A RAPID METHOD of identification and differentiation of the barbiturates by microcrystalline procedures was reported in 1956 (1). An additional report using similar methods was published (2). During the initial investigation of the identification of the barbituric acid derivatives, it was stated that work was being continued with the newer barbituric acid derivatives, the hydantoins and the nonbarbiturate sedatives. This communication covers the later work.

EXPERIMENTAL

The derivatives under consideration were isolated from the tablet, capsule, or injection in the manner described in the previous paper (1). It was found that isolation was successful by triturating the tablet and by extracting the powder with diethyl ether, if the dosage form contained only one active ingredient. Sublimation was performed on the Kofler block in addition to the melting points. It should be pointed out that the recorded melting points will vary, depending on the method used for determining melting points.

The amide or imide was fused on a microscope slide. Recrystallization from 50% or 70% alcohol and concentrated ammonia was performed on a microscope slide.

In the first report water was used as a solvent rather than 50% alcohol. However, it was found that crystallization from 50-70% alcohol was more rapid and as useful for purposes of identification. The crystalline habits from 50% alcohol were different in most cases than the crystalline habit obtained by using water as the crystallizing solvent. The habits obtained from water and acetone were similar in most cases.

The X-ray method of analysis was discussed in the first paper (1). It was stated that "the reproducibility of the angles at which the peaks occur for

the same barbituric acid derivative on various runs, as here reported, is of the order of ± 0.1 degree."

The melting points of some of the amides and imides have been published in the literature. Since the melting point varies with the apparatus used, it was decided to report the melting points of all compounds studied. These are listed in Table I.

The characteristic diffraction patterns were obtained by subjecting the powder to Cu K-alpha radiation from the X-ray spectrometer and recording the diffracted radiation on a chart, using a modified GM tube with a recording potentiometer. The d-distances were calculated and are reported in Table II. The X-ray instrument used was a Morelec (North American Phillips Co.) Geiger-Counter X-Ray Spectrometer Type No. 12012, equipped with a recording potentiometer (Brown Electronik Strip Chart Pyrometer Single Point Recorder, High Speed), using a rotating specimen holder.

DISCUSSION

A discussion of the peculiarities of the following compounds was deemed desirable.

Diphenylhydantoin.—Extraction from the tablet was easily accomplished by trituration followed by mixing with ether, decantation and evaporation of the supernatant solution. By the sublimation procedure, characteristic orthorhombic-like crystals were obtained between 163-168°. At 245°, the crystals changed to needle-like rods.

Mesantoin.—Extraction was accomplished directly from the tablets with ether. When mesantoin was dissolved in 70% alcohol and allowed to evaporate on a slide, characteristic pseudohexagons were obtained following scratching of the slide. Similar crystals were obtained by dissolving the mesantoin in 0.1N NaOH and precipitating with 1N sulfuric acid. If the slide was scratched following the addition of sulfuric acid, the size of the crystals was very small.

Methsuximide.—When methsuximide was recrystallized from 70% alcohol, pseudohexagons were obtained. The crystals became quite large when the alcohol was allowed to evaporate slowly. The "inclined faces" appeared to be beveled. The crystals were quite small and the beveling was not apparent if the solution was scratched after initial precipitation.

Milontin.—Twinning appeared to be prevalent

* Received January 6, 1958, from the pharmaceutical chemistry laboratories, School of Pharmacy, University of Southern California, Los Angeles 7, Calif.

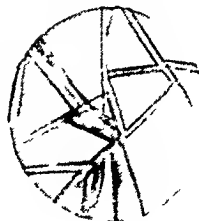
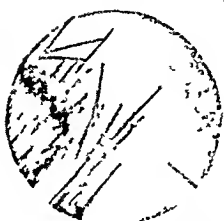
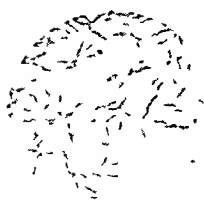
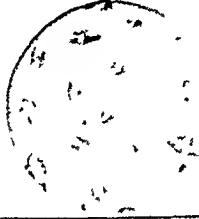
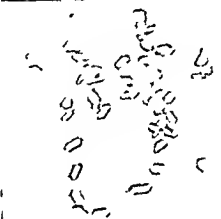
† Police chemist, Los Angeles Police Department.

The authors wish to thank Mr. John Freeman for some initial studies with the hydantoins. This study was completed in part by a grant from the University of Southern California Pharmacy Alumni Association Development Program. The following companies furnished liberal quantities for study: Abbott (Phenurone, Tridione), Parke, Davis (Dilantin), and Sandoz (Mesantoin).

SUBLIMATION

FUSION

FROM 50% ALCOHOL

FROM CONC
AMMONIADIPHEN-
YLHY-
DANTOINETHO-
TOINMESAN-
TOINMETH-
SUX-
IMIDEMILON-
TINPHFNA
CEMIDEFig. 1.—Photomicrographs showing variation in crystal habits of barbituates All magnifications $\times 45$

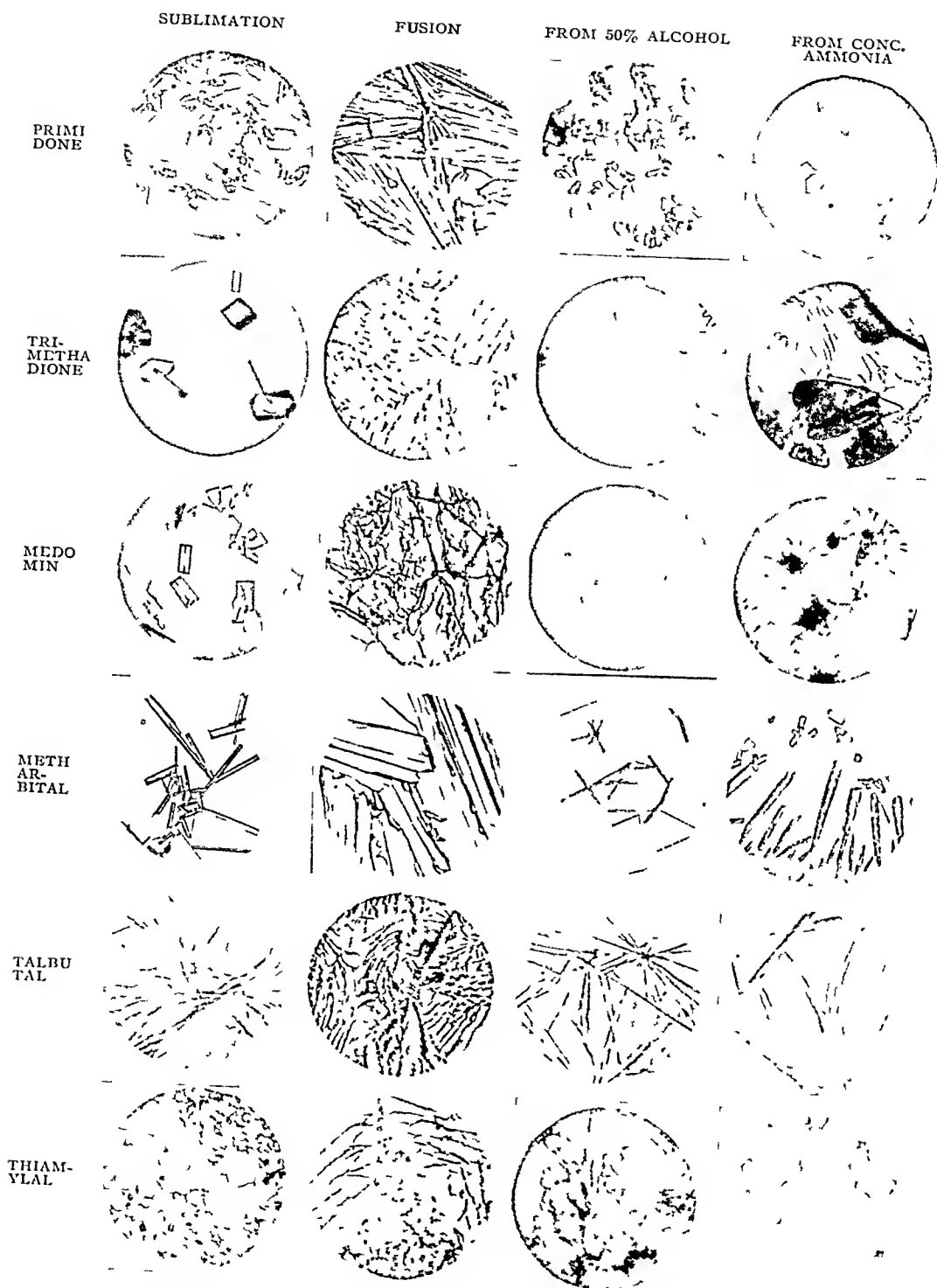


Fig 1 —Photomicrographs showing variation in crystal habits of barbituates. All magnifications $\times 45$

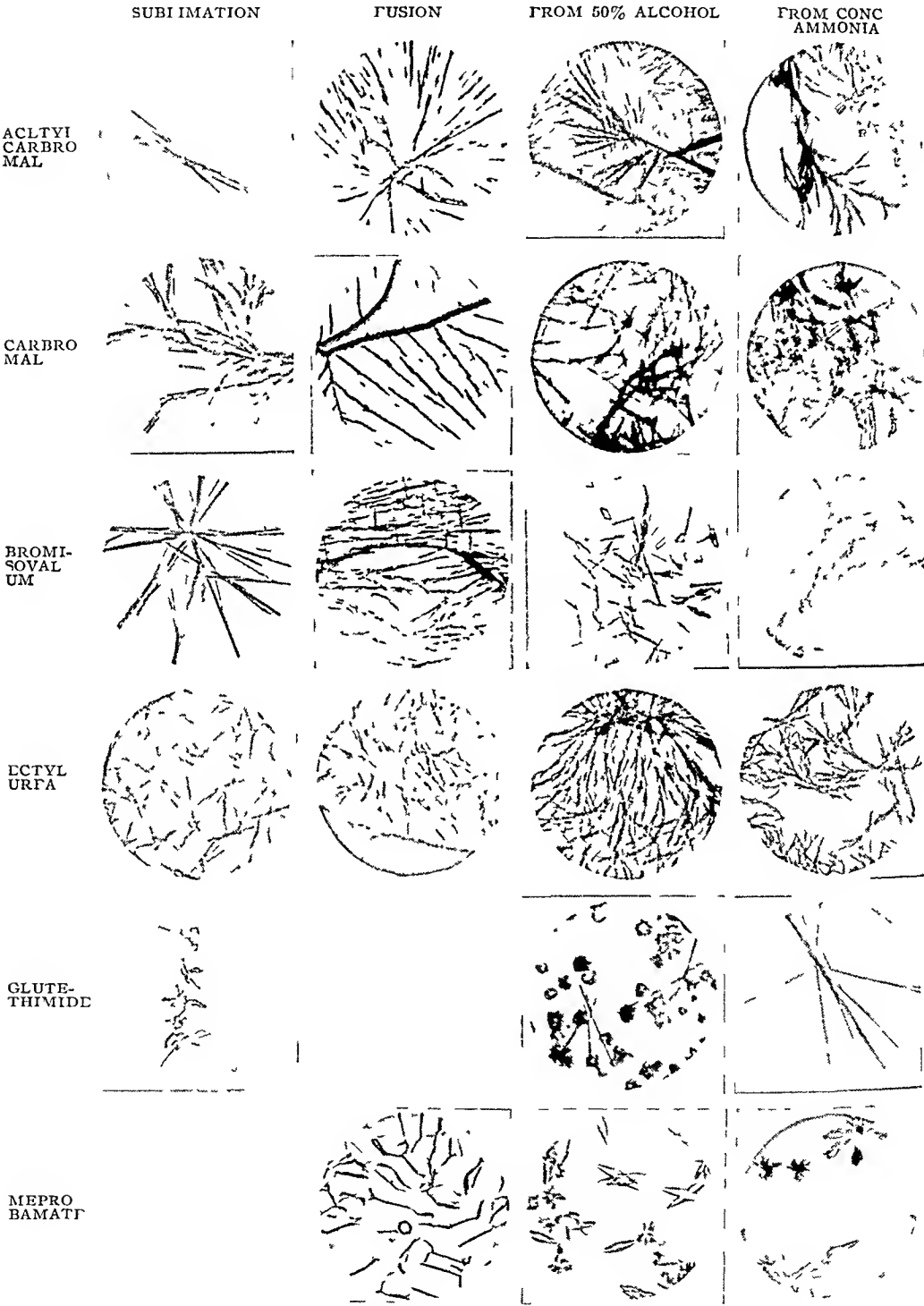


Fig 1 —Photomicrographs showing variation in crystal habits of barbiturates All magnifications X 45

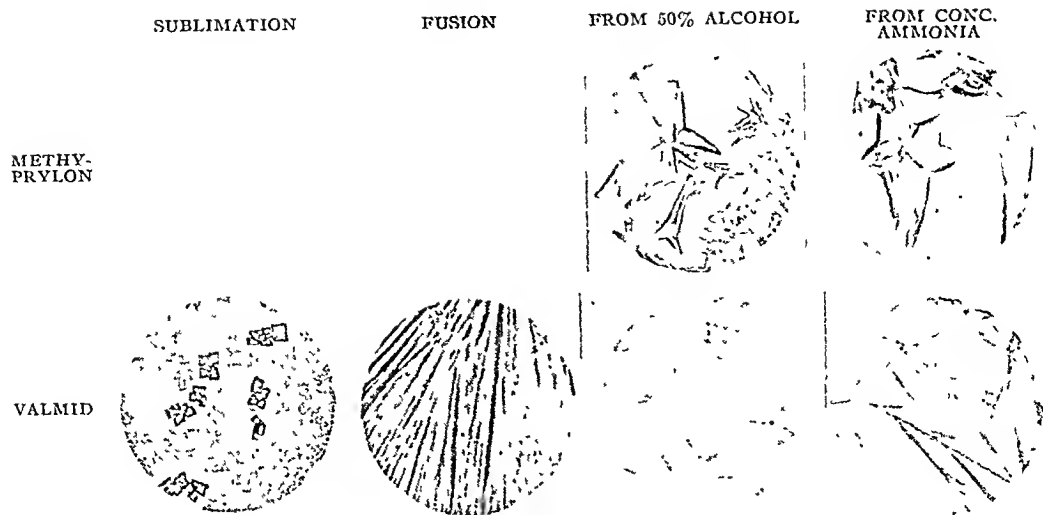


Fig. 1.—Photomicrographs showing variation in crystal habits of barbituates All magnifications $\times 45$

TABLE I.—MELTING POINTS OF THE AMIDES AND IMIDES

Compound	Chemical Name	M. p., °C.
Diphenylhydantoin	5,5-diphenylhydantoin	295-299
Ethotoin	3-ethyl-5-phenylhydantoin	90-92
Mesantoin	3-methyl-5-ethyl-5-phenylhydantoin	136-139
Methsuximide	N-methyl- α , α -methylphenylsuccinimide	52-52.5
Milontin	methylphenylsuccinimide	72-73
Phenacemide	phenylacetylurea	203-206
Primidone	5-phenyl-5-ethylhexahydropyrimidine-4,6-dione	289
Trimethadione	3,5,5-trimethyloxazoline-2,4-dione	44.5-45
Medomin	5-ethyl-5-(1-cycloheptenyl)barbituric acid	169-171
Metharbital	N-methyl-5,5-diethylbarbituric acid	152-153
Talbutal	5-allyl-5-sec. butylbarbituric acid	98-101
Thiamylal	5-amyl-5-(1-methylbutyl) thiobarbituric acid	123-130
Acetylcarbromal	N ¹ -acetyl-N-bromodiethylacetylurea	108-110
Carbromal	bromodiethylacetylurea	114-117
Bromisovalum	2-monobromisovalerylurea	153-154
Ectylurea	2-ethylcrotonylurea	189-191
Glutethimide	α -ethyl- α -phenylglutarimide	91-92
Meproamate	2-methyl-2n-propyl-1,3-propanediol dicarbamate	118-119
Methypylon	3,3-diethyl-5-methyl-piperidine-2,4-dione	76-78
Valmid	1-ethinylecyclohexylcarbamate	97-98

when the milontin was crystallized from 70% alcohol. Milontin showed no apparent solubility with 0.1N NaOH as no precipitate followed with addition of sulfuric acid. The compound was extracted also directly from the tablet with diethyl ether or chloroform. When the ether or chloroform solution of milontin was allowed to evaporate, the solid material showed a faint yellow color.

Phenacemide.—The compound was extracted directly from the tablet with ether. After evaporation of the ether, the material had the appearance of asbestos fibers. When phenacemide was sublimed, needles appeared at approximately 110°, changed to rhombic columnar crystals, with the reappearance of needles at approximately 175°.

Primidone.—As illustrated in the photographs, characteristic crystals were obtained from alcohol. Some rosettes of pseudohexagonal plates were observed.

Trimethadione.—The very low melting point was characteristic. Cooling on an ice bath was necessary for fusion studies as well as crystal studies from alcohol and ammonia. A low controlled temperature was necessary to obtain sublimed crystals.

Metharbital.—The compound was extracted directly from the solid dosage form. The crystals obtained from alcohol were characteristic since the cleavage perpendicular to the long axis was often jagged. Swastika-like crystals were often obtained by dissolving the metharbital in 0.1N NaOH and precipitating with 1N sulfuric acid; if this precipitation were performed on a slide followed by scratching, crystals were obtained which were similar to those obtained from concentrated ammonia.

Talbutal.—The characteristic crystals formed from the oily droplets in an alcoholic solution was one of the most satisfactory methods of identification.

TABLE II—THE D-DISTANCES FOR THE PURIFIED AMIDES AND IMIDES USING CU K-ALPHA RADIATION

Diphenyl hydantoin	Etho- toin	Mesantoin	Meth- suximide
7 74 ^a	10 7 ^a	6 84 ^a	10 6 ^a
6 84	5 31 ^a	5 89	6 69
5 33	4 69	5 03	6 35
5 11	3 93	4 59 ^a	6 09
4 89	3 62	4 20 ^a	5 20
4 36	3 51	3 92	5 10 ^a
3 94 ^a	3 28	3 62	4 86
3 91 ^a	3 15	3 40	3 76
3 42	3 06	3 26	3 52 ^a
3 22	2 63 ^a	2 66	3 37
Milontin	Phen acemide	Pri madone	Trimeth- adione
13 3	10 1	13 6	6 45 ^a
8 32	8 40	6 92 ^a	5 20 ^a
6 54		6 54	4 49
6 31	5 44	6 24	4 43
5 34 ^a			
5 21 ^a	4 42 ^a	5 74	3 86
4 19 ^a	4 05	4 59 ^a	3 48
3 89	3 67 ^a	4 16 ^a	3 19 ^a
3 47	3 34	3 65	2 79
3 39	3 13 ^a	3 44	2 76
	2 42	3 19	2 13
	3 02		
Medomin	Meth arbutal	Talbutal	Thiamylal
15 3	8 78 ^a	10 2 ^a	12 7 ^a
7 71 ^a	6 71 ^a	7 77	7 03 ^a
6 29	5 99	5 76 ^a	6 33 ^a
5 91	5 87	5 31	5 10
5 16 ^a	5 76 ^a	5 03	4 52
4 34	5 37	4 51	4 22
3 86	4 12	3 87	4 10
3 37			
3 00	3 50	3 57	3 79
2 81	3 29	3 52 ^a	3 52
	3 11	3 34	3 16
Acetyl carbromal	Carbromal	Bromi- sovalum	Ectylurea
6 49 ^a	9 74	13 4	6 42 ^a
6 39 ^a	8 17	6 74 ^a	5 13
4 86	7 12 ^a	5 93	4 73 ^a
4 38	6 74	4 85	4 45
4 22	4 47	4 56	3 92
3 35 ^a	4 30	4 34 ^a	3 78 ^a
3 17	3 86 ^a	3 86	3 18
2 82	3 81 ^a	3 44	2 99
2 41	3 28	3 08	2 72
2 04	3 19	2 75 ^a	2 68
Glute- thimide	Mepro- bamate	Methy- pyrilon	Valmid
10 3	14 1	6 29 ^a	12 5 ^a
7 23	8 21	6 20 ^a	5 47
6 22 ^a	6 95 ^a	5 97	5 36 ^a
6 01	4 71	5 36 ^a	5 29 ^a
5 11 ^a	3 94 ^a	4 35	4 91
4 59	3 70	4 03	4 10
3 78 ^a	3 54		3 42
3 52	3 13	3 47	3 20
3 27	2 75	3 03	3 12
3 20	2 37	8 52	3 08

^a Strong

Thiamylal.—The material was extracted directly from the solid dosage form with diethyl ether. Dendritic crystals were obtained from the alcoholic solution. Leaflets were obtained in cluster form when crystallized from concentrated ammonia. The contrast of these leaflets was very low and suitable photographs were practically impossible.

Twinned crystals (perhaps elbow twinning) were obtained when a basic solution was acidified with sulfuric acid in order to precipitate the compound.

Acetylcarbromal.—Characteristic "wheat bundles" obtained from an alcoholic solution redissolved on scratching. Rectangular plates, similar to those obtained from concentrated ammonia, were then observed. Crystals obtained by sublimation on the Kofler block were obtained more easily when a microscope slide was used rather than the hanging drop slide.

Carbromal.—When the material was sublimed, needles (sheaths) appeared at approximately 80°. As the temperature continued to rise, some rod-like forms appeared which did not melt even at 140°. This was characteristic since the melting point of carbromal was 114–117°.

Bromisovalum.—The formation of a larger number of plates, as illustrated by crystallization from 50% alcohol, was hastened by scratching the slide. Similar plates were obtained by precipitating the material from a basic solution with sulfuric acid. When the compound was sublimed, platelets were first obtained; with continued heating needles were observed.

Glutethimide.—When the tablets were treated directly with 70% alcohol, platelets were obtained. Needles were obtained in addition to the plates when the ether extract was recrystallized with 70% alcohol. As this alcohol was allowed to evaporate, crystallization continued on the platelets (pseudo-hexagons) and many varied crystalline shapes were observed. When the ether extract was dissolved in 0.1N NaOH and precipitated with 1N sulfuric acid, columnar-shaped crystals were obtained; needles were obtained on scratching the slide.

Meprobamate.—The characteristic crystals for this compound were those illustrated in the photographs from 50% alcohol and ammonia. Crystals from sublimation were not observed.

Methypyrilon.—Characteristic crystals from sublimation were not observed. When sublimation was performed on the Kofler block and the slide was observed under 260X magnification, small columnar (monoclinic ?) crystals were observed. An oil was obtained when the tablets were extracted with diethyl ether or chloroform; after standing and then scratching crystallization occurred. The crystals from 50% alcohol and concentrated ammonia were very characteristic. It was noted that the methypyrilon reacted with the lead when placed on the lead for X-ray diffraction study. It was noted also that pure methypyrilon, when treated with a drop of gold chloride in phosphoric acid (1:20 H₂AuCl₃ in [2 + 1] H₃PO₄), formed columnar crystals. A similar reaction was obtained if the reagent was added to the tablet.

Valmid.—Sublimation crystals were characteristic. When the Valmid was crystallized from alcohol, columnar crystals were observed. The columnar crystals resulted from the definite cleavage of platelets. Irregularly shaped crystals were obtained when the alcoholic solution was scratched.

REFERENCES

- (1) Penprase, W. C., and Biles, J. A., *THIS JOURNAL*, 45, 585 (1956).
- (2) Von Erich Kassau, *Deut. Apotheker-Ztg.*, 97, 135 (1957).

Separation of 1,2,3-Trimercaptopropane from Dimercaprol by Partition Chromatography*

By E. G. RIPPIE, A. A. KONDRITZER, and R. I. ELLIN

A method is presented of separating mixtures of 1,2,3-trimercaptopropane and dimercaprol by means of a partition chromatographic column with an aqueous buffer solution as the internal phase and a mixture of isopropyl ether and petroleum ether as the external phase. Dimercaprol, free of 1,2,3-trimercaptopropane, has been prepared and physical data are given.

AN EARLIER PUBLICATION from this laboratory (1) indicates the presence of a trithiol, 1,2,3-trimercaptopropane, as an impurity in every lot of 2,3-dimercaptopropanol (Dimercaprol, U. S. P. XV; British Anti-Lewisite; B.A.L.) investigated. Trimercaptopropane, on parenteral injection into rats and rabbits, has been found to be significantly more toxic than dimercaprol (2, 3).

Two objectives of this investigation were the development of a method for the separation of trimercaptopropane from dimercaprol and the subsequent preparation of dimercaprol free of the trithiol. Physical data can then be determined and more exacting specifications, including physical constants, established for dimercaprol.

EXPERIMENTAL AND RESULTS

Dimercaprol free of 1,2,3-trimercaptopropane is difficult to obtain by ordinary distillation procedures due to the closeness of their boiling points. A partition chromatography system was chosen as one most likely to assure separation of each component, as well as satisfactory recovery of the sample.

Isopropyl ether was selected for trial as the eluant because of its protophilic properties. Since the trithiol compound is more acidic than dimercaprol a solvent of this type would be expected to favor solubility of the trithiol, whereas the aqueous phase favors solubility of the dimercaprol. However, using only isopropyl ether as the eluant, complete separation was not accomplished. The addition of petroleum ether to the isopropyl ether markedly decreases the solubility of dimercaprol, whereas it affects the solubility of trimercaptopropane to a lesser extent. With a 1:1 (v/v) mixture of petroleum ether and isopropyl ether as the eluant a very good separation of the two compounds was obtained, as shown in Fig. 1.

Materials and Equipment.—Dimercaprol; 1,2,3-trimercaptopropane (4); silicic acid, Mallinckrodt, chromatography grade; chloroform, reagent grade; freshly distilled isopropyl ether; petroleum ether, washed with sulfuric acid and distilled between 35–50°; 0.2 *M* phosphate buffer of pH 6, containing 0.1% sodium bisulfite; standard 0.1 *N* iodine solu-

tion. Small chromatographic tubes: 12 to 13 mm. internal diameter (I.D.) \times 600 mm., constricted at one end into a short delivery tube, 5 mm. I.D. Indentations were made near the lower end of the tube to support the silicic acid column. A glass rod, flared and ground to fit the tube, was used for packing the column. Large chromatographic tube: 86 mm. I.D. \times 750 mm., constricted at one end into a short delivery tube of 15 mm. I.D. and with a sintered glass plate of coarse porosity above the delivery tube to support the silicic acid column. The column was packed by means of a wooden rod fitted at one end with a wooden disk having a diameter slightly less than the internal diameter of the tube.

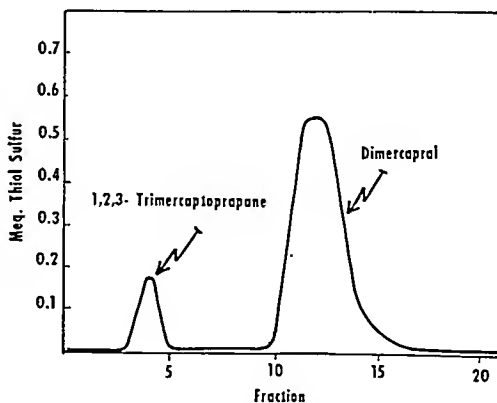


Fig. 1.—Partition chromatogram of a mixture of 1,2,3-trimercaptopropane and dimercaprol. Phosphate buffered solution was used as the internal phase; petroleum ether-isopropyl ether mixture (1:1 v/v) was used as the eluant. Fractions were 3 ml. in volume.

Preparation of Column.—Twenty grains of silicic acid were thoroughly mixed with 20 ml. of the phosphate buffer containing bisulfite. The mixture was then made into a slurry by mixing with 100 ml. of chloroform. Successive portions of the slurry were poured into a small chromatographic tube, at the bottom of which had been placed a small plug of

* Received January 27, 1958, from the Physiology Division, Directorate of Medical Research, Army Chemical Center, Md.

glass wool. After each addition of the slurry the silicic acid was firmly packed by means of the ground glass plunger. A layer of liquid was always kept above the column to prevent the formation of air spaces. The column, usually 25–30 cm. in height, was washed free of chloroform with the 1.1 (v/v) petroleum ether-isopropyl ether solvent.

Procedure.—The solvent head was allowed to fall to the level of the silicic acid. Approximately 100 mg of dimercaprol contained in two ml. of petroleum ether-isopropyl ether was placed on the column and the liquid was again allowed to fall to the level of the silicic acid. The walls of the column were rinsed with a 2-ml portion of solvent. The rinsing was allowed to pass into the column before the chromatographic tube was filled with solvent. Three-ml. fractions were collected and analyzed for thiol sulfur content by titration with standard iodine and the elution curve determined (Fig. 1). A two-ml aliquot of the original sample, titrated directly with standard iodine solution, served as a check on the quantitative recovery of total sample.

Analysis of BAL by Partition Chromatography.—Four experimental samples were prepared by dissolving dimercaprol and trimercaptopropene in the ether solvent mixture. Aliquots containing approximately 100 mg of dimercaprol and an amount of trithiol ranging from 10 to 55 mg were analyzed by the above procedure. The results, in Table I, show that approximately 98% of the trimercaptopropene and 92–96% of the dimercaprol were recovered. On passing through the column both components are probably partially oxidized. Barron, *et al* (5), reported that trimercaptopropene and dimercaprol were readily oxidized in the presence of trace amounts of copper, dimercaprol being oxidized twelve times as rapidly as trimercaptopropene. The silicic acid and buffer salts have maximum limits of heavy metal impurities that suggest the presence of catalytic quantities in the column.

Analysis of laboratory and commercial lots of dimercaprol was carried out by taking three successive fractions (a) a 20-ml fraction containing

the trimercaptopropene impurity; (b) a 3-ml. fraction which served as a check on the separation of the two components; and (c) a 40-ml. fraction containing the dimercaprol. Results are given in Table II.

Preparation of Dimercaprol Free of Trimercaptopropene.—With the large chromatographic column, using fifty times the quantity of silicic acid, dimercaprol, and other reagents used in the above procedure, the same method of isolation was followed. The portion of eluate containing dimercaprol was carefully evaporated and the dimercaprol distilled under reduced pressure, b. p. 66–68°/0.2 mm Hg. The refractive index was determined with an Abbe refractometer; $n_D^{25} = 1.5647$. The thiol sulfur content was 51.8%; theory, 51.6%.

TABLE II.—RECOVERY OF 1,2,3-TRIMERCAPTOPROPANE FROM COMMERCIAL LOTS OF DIMERCAPROL

Sample No	Weight, mg	Trithiol Containing Impurity Found	
		mg	Per Cent
1	94.4	1.37	1.45
2	91.6	1.54	1.68
3	112.1	5.29	4.72
4	96.5	8.06	8.35
5	100.1	5.96	5.95
6	116.4	7.38	6.34
7	91.7	2.60	2.84
8	91.0	0.66	0.73
9	94.5	3.78	4.00
10	98.7	2.59	2.63
11	116.9	3.88	3.32
12	102.5	4.29	4.19

SUMMARY

A partition chromatography method of separation of 1,2,3-trimercaptopropene from dimercaprol on a laboratory scale has been presented. The method has been applied to the estimation of the trithiol present in various laboratory and commercial preparations of dimercaprol. Dimercaprol, free of trimercaptopropene, has been prepared and some physical constants determined.

REFERENCES

- (1) Ellin, R. I., and Kondritzer, A. A., *THIS JOURNAL*, 47, 12 (1958).
- (2) Fitzhugh, O. G., Woodward, G., Braun, H. A., Lusby, L. M., and Calvery, H. O., *J. Pharm. Exptl. Therap.*, 87, (Supplement), 23 (1956).
- (3) Unpublished data (1957), this laboratory.
- (4) Miles, L. W. C., and Owen, L. N., *J. Chem. Soc.*, 1950, 2942.
- (5) Barron, E. S. G., Miller, Z. B., and Kalnitsky, G., *Biochem. J.* 41, 62 (1947).

TABLE I.—EFFICIENCY OF SEPARATION OF DIMERCAPROL AND 1,2,3-TRIMERCAPTOPROPENE BY PARTITION CHROMATOGRAPHY

Sample	Component ^a	Amt in Weighed Sample, ^b mg	Amt Found, mg	Recovery, %
1	A	54.5	52.9	97.1
	B	100.8	92.9	92.2
2	A	28.1	27.9	99.1
	B	100.3	92.3	92.0
3	A	17.8	17.5	98.4
	B	85.5	82.7	96.7
4	A	10.3	10.1	97.9
	B	107.8	99.5	92.3

^a Components are designated as A, 1,2,3 trimercaptopropene, and B, dimercaprol.

^b Corrected for percentage of trithiol already present in dimercaprol sample used.

Bactericide/Leukocide Ratio: A Technique for the Evaluation of Disinfectants*

By LEO GREENBERG and JAMES W. INGALLS

A rapid and simple method for the evaluation of antiseptic substances, using animal leukocytes is described. A comparison between the bactericidal and leukocidal concentrations of a given compound yields a relative measure of the toxicity of the test substance, and under the test condition, only gentian violet, Chlorox, and Isodine, of fifteen compounds tested, show a lower toxicity to phagocytes than to bacteria. The method described should prove valuable for small laboratories or for rapid evaluation of new compounds, although values so obtained cannot be the sole criterion for the determination of the effectiveness or usefulness of a wound disinfectant.

SINCE 1931, the testing of disinfectant substances in the United States has largely been carried out by the U. S. Food and Drug Administration method, commonly referred to as the phenol coefficient technique. Results obtained by this method have been seriously criticized on several grounds, not the least of which is the presumed lack of practicality.

To meet this criticism, a number of *in vivo* testing methods have been devised for use in the evaluation of germicidal materials, some of the best known being the mouse tail amputation method (1), the white mouse skin test method (2), and the chorionic-allantoic egg membrane method (3). Since these methods in turn yield little information concerning the relative toxicity of the compound being tested, still other evaluation procedures have been suggested. These include the use of embryonic chick heart fibroblasts (4-7), the abdominal wall of white rabbits (8), rabbit spleen cells (9), spindle cell and periosial cell cultures (10), and manometric measurements of mouse liver cells (11).

Nye (12) in 1937 proposed that the toxicity of antiseptics be determined against leukocytes, and a complex technique for such determinations has been developed by Welch, *et al.* (13, 14). This technique involves the preparation of artificially opsonized staphylococci, special rotating machinery and an incubation period beyond the time in which antiseptics are normally in active contact with tissues. Since these studies, little attention has been paid by research workers to the use of white blood cells as a substrate for the measurement of chemical toxicity, and at least part of this apathy may be ascribed to the complexity of the testing procedures involved.

Since leukocytes represent a readily available source of cellular material, they would appear to be a more suitable source of tissue cells for use in routine analytical procedures than embryonic chick heart fibroblasts or other materials used in toxicity testing. Moreover, leukocytes themselves represent a major part of the infection process, a process which disinfectant substances are designed to influence favorably. A substance which significantly affects the phagocytic process affects the body's defense against pathogenic microorganisms, and a germicide whose toxicity to this vital cellular defense mechanism is far greater than its toxicity to bacterial pathogens may reasonably be expected to alter, to a greater or lesser degree, the body's resistance to bacterial invasion.

In view of this, the purposes of this investigation were first, to develop a simplified method for the determination of chemical toxicity to leukocytes which might routinely be used by small laboratories for the evaluation of wound disinfectants, and secondly, to determine through the use of such a technique, the bactericidal and leukocidal values of some of the most commonly used wound disinfectants (several selections are of historical rather than current interest).

METHODS AND MATERIALS

Commercial disinfectants used in this study were purchased locally or prepared with reagent grade ingredients. Dilutions were prepared with sterile pipets and glassware using sterile isotonic saline. The bacterial culture used was a stock culture of *Micrococcus pyogenes* var. *aureus* (209P) originally obtained from the American Type Culture Collection and maintained by serial transfer on FDA agar slants. As needed, a small loopful of surface growth was transferred to a tube of sterile FDA broth and incubated at 37° for six hours. One loopful of this broth was then inoculated into fresh sterile FDA broth and incubated at 37° for eighteen hours.

* Received November 21, 1957, from the Departments of Microbiology and Pharmacology, Brooklyn College of Pharmacy, Long Island University, Brooklyn, N. Y.

Preliminary study showed that such broth tubes contained approximately 300,000,000 organisms per ml. regardless of the size of the initial agar slant inoculum.

Bactericidal values were determined by pipetting 0.2 ml. of eighteen-hour broth culture of the test organism into sterile Wasserman tubes and adding 1.0 ml. of the appropriate dilution of the disinfectant at 37°. The contents of the tubes were well mixed and kept for exactly ten minutes at 37° after which a 4 mm. loopful was removed and transferred to tubes containing 2.0 ml. of sterile FDA broth. In the case of mercurial disinfectants, thioglycollate broth was used to eliminate the bacteriostatic effect of the metal. All runs were done in duplicate and checked a week later to verify results obtained.

For testing leukocyte toxicity, fresh blood was obtained by cardiac puncture or from the vena cava of young male rats under light ether anesthesia and defibrinated by whipping gently. Routine white blood cell counts were done to eliminate grossly abnormal animals; counts on blood so obtained averaged slightly below published normals for rat blood, presumably due to the loss of some leukocytes in the removal of the clot. It should be pointed out that rat blood was chosen for use in this study only because of its availability; random observations indicated that human, guinea pig, or rabbit leukocytes each yield consistent values, although such values differ from species to species.

One-fifth milliliter of blood was pipetted into sterile 50-ml. centrifuge tubes and 1.0 ml. of the appropriate dilution of the disinfectant at 37° added. Tubes were well mixed and maintained at 37° for exactly ten minutes. Approximately 50 ml. of warmed sterile isotonic saline was added, stirred, and immediately centrifuged for thirty seconds at high speed, the supernatant fluid being drawn off and discarded. This washing was repeated twice more to remove all traces of the disinfectant.

Survival of leukocytes was measured by the ability of polymorphonuclear neutrophils to phagocytize a bacterial suspension prepared by adding 1.0 ml. of eighteen-hour broth culture of *M. aureus* to

3.0 ml. of sterile rat serum and incubating for at least four hours prior to use. One-tenth milliliter of this suspension was added to the cellular sediment in the centrifuge tubes, mixed well, and incubated at 37° for one-half hour. After this time, a loopful of the mixture was removed, smeared on sterilized glass slides, stained with Wright's stain for one minute, buffered with Wright's buffer for four minutes, rinsed in tap water for thirty seconds, dried, and examined under oil immersion. Ten typical polymorphonuclears were counted for bacterial inclusions, a total ingestion of ten or more bacteria in the cells counted being taken as an indication of phagocytosis, and hence, survival. Normal controls were run simultaneously with each set of leukocidal values to verify the phagocytic powers of the blood being used.

RESULTS AND DISCUSSION

The bactericidal values for the fifteen disinfectants tested are listed in column 1 of Table I. Since the dilutions which were used in the evaluation of these substances varied, the highest determined concentration in which growth was obtained is also listed (column 2). It seems reasonable to suppose that were additional dilutions made in the range between the two determined values, the bactericidal point would be slightly altered in some cases.

Inspection of the data reveals that gentian violet, tincture of Zephiran, tincture of iodine, and tincture of Merthiolate rank highest in germicidal efficiency of the pure compound. That these latter results are not due to the effects of the alcoholic vehicle can likewise be determined. The complete ineffectiveness of tincture of green soap and mercurochrome to show germicidal activity under conditions of this technique would appear to be somewhat surprising, as is the relatively poor showing of bichloride of mercury. If this table is viewed in the light of commonly used strengths of these disinfectant substances, it would appear that Lysol, Clorox, gentian violet are most germicidal, followed closely by tincture of iodine, Isodine, and tincture of Zephiran in descending order.

TABLE I.

Compound	Bactericidal Dilution	Growth Dilution	Leukocidal Dilution	Phagocytic Dilution	B/L Ratio
Clorox	1:100	1:150	1:50	1:100	0.5
Ethanol (95%)	F. S. ^a	1:2	1:5	1:10	5
Green Soap Tincture	F. S. I. ^b	F. S. ^a	1:500	1:1,000	...
Gentian Violet	1:10,000	1:15,000	1:4,000	1:5,000	0.4
Hydrogen Peroxide (6%)	F. S. ^a	1:2	1:10	1:20	10
Iodine Tincture	1:70	1:85	1:2,000	1:5,000	28.6
Isodine	1:55	1:70	1:50	1:100	0.9
Lysol	1:100	1:150	1:3,000	1:5,000	30
Mercuric Chloride	1:100	1:200	1:20,000	1:30,000	200
Mercurochrome	F. S. I. ^b	F. S. ^a	F. S. I. ^b	F. S. ^a	...
Merthiolate Tincture	F. S. ^a	1:2	1:20	1:50	20
Phenol (abt. 90%)	F. S. ^a	1:10	1:1,000	1:2,000	1,000
Potassium Permanganate	1:100	1:200	1:2,000	1:5,000	20
S. T. 37	1:2	1:4	1:2	1:5	1
Zephiran Tincture	1:10	1:25	1:50	1:100	5

^a F. S. = full strength. ^b F. S. I. = full strength ineffective.

The leukocidal concentrations of the disinfectants tested are listed in column 3, and the maximum tested concentration of the compound permitting phagocytosis is likewise indicated. Again it should be pointed out that were determinations made in the gap between these two values, the leukocidal point would probably shift in certain cases.

Column 5 indicates the bactericidal/leukocidal ratio of each of the compounds tested. Theoretically as with all toxicity indexes, the lower the ratio, the safer the compound for routine use as a wound disinfectant. The relatively high value obtained for iodine tincture contrasts sharply with the lack of cellular toxicity previously reported (13), and the result for Clorox does not bear out previous reports of high leukocyte sensitivity to chlorine compounds (12). As the results indicate, only gentian violet, Clorox, and Isodine are less toxic to white blood cells than to bacteria, while classic disinfectant substances such as phenol and mercuric chloride evidence inordinate leukocidal properties.

However, the limitations of this and other toxicity studies must be carefully identified. Under practical conditions of disinfection, there is little doubt that phenol is effective, despite its evident cellular toxicity. Moreover, it would appear quite unlikely that the local destruction of phagocytic cells in a wound will long affect the healing process, since for all practical purposes the body's supply of leukocytes is unending. It has even been shown (15) that the presence of mercuric chloride may actually raise rather than lower the ability of phagocytes to engulf bacteria. Thus, the technique described here is not a substitute for clinical evaluation nor an infallible guide to the selection of nontoxic disinfectants. It is rather to be regarded as a simplified and rapid method for obtaining one small part of the profile of potential disinfectant agents, in

the final analysis, it is the entire profile on which the use or non-use of the compound tested must be based.

SUMMARY

1. A rapid and simplified modification of earlier techniques for the comparison of bactericidal and leukocidal values of wound disinfectants has been described.

2. Under the test conditions, only gentian violet, Clorox, and Isodine, of the disinfectants tested, are less toxic to leukocytes than to bacteria.

3. The limitations on the use and significance of the test procedure have been discussed.

REFERENCES

- (1) Nungester, W. J., and Kempf, A. H., *J. Infectious Diseases*, 71, 174 (1942)
- (2) Sarber, R. W., *J. Pharmacol. Exptl. Therap.*, 75, 277 (1942)
- (3) Green, T. W., and Birkeland, J. M., *J. Infectious Diseases*, 74, 32 (1944)
- (4) Salle, A. J., and Lazarus, A. S., *Proc. Soc. Exptl. Biol. Med.*, 32, 665 (1935)
- (5) Salle, A. J., McOmie, W. A., and Schechmeister, I. L., *J. Bacteriol.*, 34, 267 (1937)
- (6) Salle, A. J., McOmie, W. A., Schechmeister, I. L., and Foord, D. C., *Proc. Soc. Exptl. Biol. Med.*, 37, 694 (1938)
- (7) Salle, A. J., *Appl. Microbiol.*, 3, 63 (1955)
- (8) Birkhaug, K. E., *J. Am. Med. Assoc.*, 95, 917 (1930)
- (9) Lambert, R. A., and Meyer, J. R., *Proc. Soc. Exptl. Biol. Med.*, 23, 429 (1926)
- (10) Bucksbaum, R., and Bloom, W., *Proc. Soc. Exptl. Biol. Med.*, 28, 1060 (1931)
- (11) Bronfenbrenner, J., Hershey, A. D., and Doubly, J., *Proc. Soc. Exptl. Biol. Med.*, 38, 210 (1938)
- (12) Nye, N. R., *J. Am. Med. Assoc.*, 108, 280 (1937)
- (13) Welch, H., and Hunter, A. C., *Am. J. Public Health*, 30, 129 (1940)
- (14) Welch, H., Slocum, G. G., and Hunter, A. C., *J. Lab. Clin. Med.*, 27, 1432 (1942)
- (15) Lambdin, S., and Desvignes, A., *Compt. rend. soc. biol.*, 146, 1920 (1952)

Notes

A Note on a New, Versatile, Photoelectric Cell Drop Counter*

By RICHARD F. CHILDS and ALBERT L. PICCHIONI

THE NEED for an efficient, inexpensive, drop counter is frequently encountered in the laboratory. Although many instruments designed to count drops have been reported, the majority of them when used are limited with respect to such factors as rate

of falling drops, viscosity, and electrolyte content of the fluid. The photoelectric cell counter, such as that employed with chromatographic fraction collectors, does not possess these limitations; however, commercially available productions are expensive. In addition, it has been the experience of one of the authors that some of these counters require considerable adjustment before and during use in order to

* Received January 14, 1958, from the College of Pharmacy, University of Arizona, Tucson.
Presented at the Section on Pharmacy, A A A S, Indianapolis meeting, December, 1957.

insure that the drops fall directly into the light beam impinging on the photocell.

Through the use of an inexpensive, miniature, transistor photodiode, and a new principle for activating this photocell, an economical, highly efficient drop counter has been produced. The instrument consists of two separate units—the "Sensor" and "Recorder." As illustrated in Fig. 1, the former is comprised of a cadmium sulfide photodiode, a glass dropper tube, and oblique and frontal light sources consisting of 3-volt flashlight bulbs each of which is coated in such a manner as to form a small slit yielding a narrow beam of light. The oblique light beam is directed at a 60° angle to strike beneath the photocell. When the parallel light source is used, the light beam is intentionally made to strike the small photodiode window. The Recorder unit

R-1 of the Recorder unit. The starter anode (grid) of VT-1 in the Recorder unit obtains its voltage from a voltage divider comprised of R-2, the photodiode, and R-4. When the window of the photodiode is dark, the resistance of the voltage divider is high and the starter anode voltage too low to allow VT-1 to draw plate current. When light strikes the window, however, the resistance of the photodiode drops, thereby increasing the voltage across R-4. At this time VT-1 conducts plate current through the spring-loaded relay counter. Occasionally, it is necessary to count drops of an opaque liquid. In such cases, the frontal light source is selected by adjusting SW-2 of the recorder unit. An opaque drop falling from the glass tube interrupts the light beam directed into the window of the photodiode causing a marked light intensity differential which activates the cell.

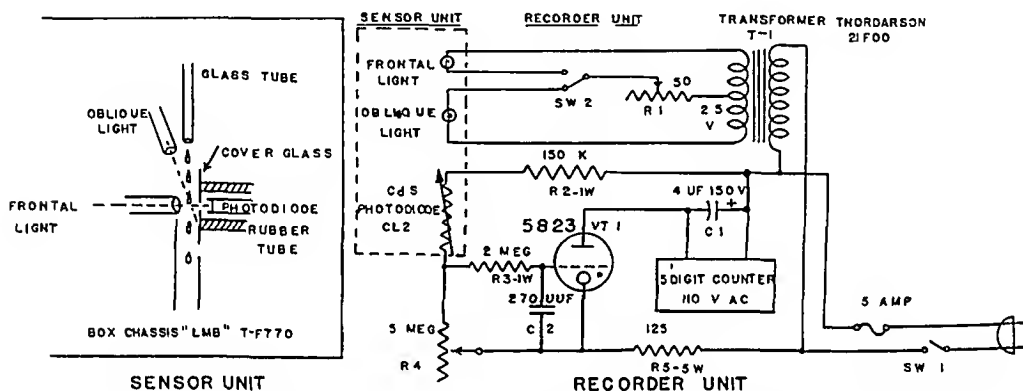


Fig. 1—Sketch illustrating relationship of elements of the Sensor unit on the left and schematic diagram for the complete photoelectric cell drop counter, including the Sensor and Recorder units, on the right.

consists essentially of a R.C.A. No 5823 cold cathode thyratron tube (VT-1) and a Mercury 110 volt a.-c. relay counter, which has been modified by attaching a Walsaco No. 7400F dial drive spring to the ratchet arm to facilitate operation of the counter directly from the plate current of VT-1.

By referring to Fig. 1, the principle of operation of both units of the drop counter can be explained. When a transparent or translucent liquid drops from the glass tube in the Sensor unit, light rays from the oblique light beam pass through each drop and are refracted. The result is a magnified bright circle of light which sweeps the small photodiode window. This sharp contrast of lighting is very effective for activating the cell. The optimum oblique light intensity for a given liquid is calibrated by adjusting

A desirable feature of this instrument is the close proximity and permanent positioning of the components of the Sensor unit (Fig. 1). Such an arrangement obviates the need for repeated adjustments before and during use of the drop counter. The Sensor unit is also designed so that the drops may be collected in a graduate for volume measurements if desired. Further, the instrument is applicable for measuring fluid inflow, since the drops passing through the Sensor unit are neither mechanically damaged nor chemically contaminated.

The drop counter is capable of accurately recording drops of liquids at rates varying from 0 to 700 per minute. The authors have found the instrument very satisfactory for determining the rate of perfusate flow in such studies as coronary perfusion of the Langendorff heart preparation.

Note on Sensitivity of the Isolated Perfused Rat Uterus to Serotonin*

By JAMES L. LEITCH, DAVID MARYN, and JEANETTE B. MOORE

GADDUM, *et al.* (1), and Amin, *et al.* (2), used the atropinized estrous uterus of the rat as a sensitive organ for the bioassay of serotonin (5-hydroxytryptamine). Barkhan (3) correlated the uterine spontaneous activity with the vaginal smear cytology of Long and Evans (4). He found that spontaneous activity was at a minimum and sensitivity to serotonin at a maximum during Stage II of the estrous cycle. Leitch, *et al.* (5), used the same vaginal smear technique to determine the stage of the estrous cycle in rats prior to sacrifice.

In an attempt to prolong the period of sensitization, Sprague-Dawley rats were injected subcutaneously with a microcrystalline estradiol suspension (Estrol A. S., Trico Pharmaceutical Co., Los Angeles) in doses of 100 to 600 μ g. per rat. In over 60 rats sacrificed at various times after such injection, uteri suitable for bioassay were obtained even though

According to Long and Evans (4), the vaginal smear cytology is directly correlated with uterine condition, Stage II being characterized by (a) maximal swelling of vaginal orifice, (b) cornified cells in the vaginal smear, and (c) maximal uterine distention (5 mm). The data in Table I show that after pretreatment with estradiol the condition of the uterus is not specifically indicated by the vaginal smear cytology. Although the vagina apparently continues through successive stages of the cycle, the uterine horns, as indicated by their degree of distention, are maintained in Stage II, for three and possibly four days after the injection. Swelling of the vaginal orifice seems to follow more closely the uterine condition. This swelling, rather than the vaginal smear cytology, might be more useful as a gross external sign for determining whether or not a given rat would be suitable for serotonin bioassay.

TABLE I.—REPRESENTATIVE DATA ON THE RELATIONSHIP OF ESTROUS CYCLE AND SENSITIVITY OF RAT UTERUS TO SEROTONIN

Rat Weight, Gm	Dose Estradiol, μ g.	Time Used, Days ^a	Estrous Cycle Data		Horn Diam, mm	Stage	Sensitivity to Serotonin
			Vaginal Swelling	Smear Cytology			
197	600	4	IV or interval	Interval	2	... ^b	Unsatisfactory
222	600	1	II	II	5	II	4 to 8 ng.
248	600	2	II	III	6	II	4 to 8 ng.
263	600	3	II	IV	7	II	4 to 8 ng.
263	600	4	II	Interval	8	II	4 to 8 ng.
286	400	1	II	I	3-4	II	4 to 8 ng.
218	400	2	II	II	4	II	4 to 8 ng.
225	400	4	II	IV	4	II	4 to 8 ng.
213	400	4	I or IV	Interval	2	... ^b	Unsatisfactory
257	200	1	I or IV	II	2	... ^b	Unsatisfactory
213	600	1	II	II	4	II	4 to 8 ng.

^a Number of days from the day of injection of estradiol

^b The small diameter of the uterine horns indicates that the cycle is definitely not in Stage II but is insufficient evidence to determine which of the other stages is involved

the vaginal smear cytology did not indicate Stage II of the estrous cycle. Representative data are summarized in Table I. Similar results were also obtained using diethylstilbesterol (tested only at 100 μ g. per rat). It was also noted that (a) the stage of estrus at the time of estradiol injection did not significantly affect the sensitization of the uteri to serotonin, (b) rats weighing 100 to 350 Gm. gave similar results, and (c) spontaneous contractions and also an unsatisfactory sensitivity were associated with uteri of less than 3 mm. in diameter.

To summarize, it has been found that 200-600 μ g. of estradiol per rat will sensitize the uterus to serotonin for three or four days. Although the uterus is maintained in Stage II of the estrous cycle this stage is not indicated by the vaginal smear cytology. For highest sensitivity in the serotonin assays, uterine horns, *in situ*, should be 5 mm. in diameter, regardless of the vaginal smear cytology.

REFERENCES

* Received December 23, 1957, from the Atomic Energy Project, University of California, Los Angeles 24.
This article based on work performed under Contract No AT(04-1)-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles

- (1) Gaddum, J. H., Hebb, C. O., Silver, A., and Swan, A. B., *Quart. J. Exptl. Physiol.*, **38**, 255(1953).
- (2) Amin, A. H., Crawford, T. B. B., and Gaddum, J. H., *J. Physiol. (London)*, **126**, 596(1954).
- (3) Barkhan, P., *S. African J. Med. Sci.*, **19**, 167(1954).
- (4) Long, J. A., and Evans, H. M., *Mem. Univ. Calif.*, **6**, (1922).
- (5) Leitch, J. L., Maryn, D., Debey, V. G., and Haley, T. J., *J. Pharmacol. Exptl. Therap.*, **120**, 408(1957).

Book Notices

Pharmacology for Medical Students in Tropical Areas By ROGER A. LEWIS The Popular Book Depot, Bombay, India, 1957 xx + 524 pp 13.5 x 21.5 cm Price Rs 15

This book presents the study of pharmacology with the special considerations required of the medical practitioner in tropical, underdeveloped areas, noting the different diseases and the varying forms of other diseases such as diabetes, that occur in those areas. The type of remedy may have to differ from what which is available in industrial centers. The doctor may have to dispense drugs rather than be able to refer the patient to a "corner drugstore." And the slighter or smaller stature of his patients will require dosage adjustments.

Following a brief, 56 page review of general pharmacology, the text deals with Chemotherapy of infectious disease, drugs acting on and affecting various systems of the body, and includes appendices giving simple exercises in practical pharmacy, and in experimental pharmacology. The exercises in practical pharmacy are correctly listed as such in the table of contents, but are incorrectly described as pharmacology by the headings in the appendix.

The four page section devoted to prescription writing lists the following examples of useful prescriptions: Neosynephrine hydrochloride 0.25%, Yellow oxide of mercury in simple eye ointment 1%, APC capsules, benzoic and salicylic acid ointment, Aureomycin hydrochloride 250 mg capsules, an ammonium chloride, wild cherry syrup, and glycyrrhiza fluidextract cough syrup and codeine phosphate syrup with wild cherry syrup for a productive cough. As a specialized text for a particular area, this book will undoubtedly be very useful.

Psychopathic Personalities By HAROLD PALMER Philosophical Library, N Y, 1957 179 pp 14 x 21 cm Price \$4.75

This book presents clinical essays on the study of mental ill health and purposes to achieve a mode of presentation of psychiatry acceptable to workers in other disciplines including those concerned with the management of human beings. The text takes up Psychopathic personalities, Schizophrenia, Depressive states, Obsessions, Hysteria, The epilepsies, Tension syndromes, Paranoid states, and Mania. An index is appended.

From Sterility to Fertility: A Guide to the Causes and Cure of Childlessness By ELLIOT E. PHILIPP Philosophical Library, New York, 1957 120 pp 12.5 x 18.5 cm Price \$4.75

The author, a gynecologist and obstetrician, observes that this book, in the hands of the laity, should not and cannot replace the doctor in dealing with infertility. The book gives no certain answer

to the question of infertility, but, intelligently used, it should be able to save lengthy explanations by the physician as to what he is setting out to do, and it should save the patients' having to ask questions that occur to them perhaps fifteen minutes after leaving the physician. The text is simply and clearly written, with several diagrammatic drawings as aids, and with discussions of different types of cases.

Quantitative Pharmaceutical Chemistry 5th ed By GLENN L. JENKINS, JOHN E. CHRISTIAN, and GEORGE P. HAGER The Blakiston Division, McGraw Hill Book Co., Inc., N Y 1957 xviii + 552 pp 13.5 x 20.5 cm Price \$8.50

This excellent combination textbook and laboratory manual has been revised to bring the procedures and discussions into conformity with the methods in the latest U.S.P. (XV) and N.F. (X). This fifth edition follows the design and style that have characterized the earlier editions of this widely accepted textbook, the fourth edition of which was reviewed in *THIS JOURNAL*, 42, 757(1953). The clarity that has featured the explanations of theoretical concepts and practical applications is in evidence in the new chapters on chromatography and radioactivity under physicochemical methods of analysis. The book is specifically designed for the instruction of pharmacy students and it covers the official quantitative and physical analytical methods, classifies the official procedures according to type methods of analysis, and presents some generally applicable, nonofficial methods of analysis.

This book is a very good reference for the pharmaceutical testing laboratory as well as being extremely well suited as a textbook. The format, type, printing, and binding are particularly good, and the appended index helps the reviewer locate specific procedures.

Cahiers de Synthèse Organique, Méthodes et Tableaux d'Application Vol. I By JEAN MATHIEU and ANDRÉ ALLAIS with the collaboration of J. VALLS and P. POIRIER Published under the direction of Léon Velluz. Maison et Cie, Paris, 1957 vi + 232 pp 15 x 22 cm Price 3,000 fr

This volume (in French) covers the fixation of a functional carbon on an aliphatic chain and on an aromatic nucleus. Variations of these two main classifications are discussed and illustrated, and tabulations of many compounds show starting materials, products obtained, agents of condensation and solvents, and yield. The text is liberally documented. A table of the functional groups and arrangements created is appended. The table of contents is given at the end of the book. Libraries used by synthetic organic chemists will want this book.

Scientific Edition

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Antibacterial and Antifungal Properties of β -Naphthol Derivatives V*

By R. S. BAICHWAL,† MALATI R. BAICHWAL,† and M. L. KHORANA‡

A detailed study was made on some derivatives of β -naphthol which have emerged as active antibacterial and antifungal compounds from a preliminary screening already reported. This included the evaluation of penetration power, influence of organic matter, and fungicidal activity. Further tests were carried out on a selected group of compounds, and covered such determinations as topical irritation and interference in the process of wound healing. The LD₅₀ by intraperitoneal administration of 6-hexyl- β -naphthol was found to be 127 mg. per Kg. A potentiation of fungistatic action was observed with a few naphthol derivatives in formulations containing known anti-infective agents. All compounds seemed to possess good penetration power even in ointment vehicles and were nonirritant to the intact skin of rabbits. The presence of organic matter did not appear to curtail the activities of these compounds seriously and the compounds themselves did not seem to have any adverse effect on the process of wound healing. The results obtained from these limited tests indicated that the compounds may prove useful as anti-infective agents and hence seem worthy of further toxicity and clinical studies.

PRELIMINARY SCREENING of a large number of derivatives of β -naphthol for antibacterial and antifungal activities showed some compounds to exhibit preferential activity against Gram-positive bacteria and human pathogenic fungi (1). The results suggested further studies on their anti-infective properties under a variety of test conditions simulating practical conditions of use. The selected compounds were evaluated as to methods of sterilization, penetration power, influence of organic matter, irritation on intact skin, interference in healing of wounds, anti-inflammatory property, and acute toxicity. Further, the antifungal compounds were tested

for their fungicidal activity, and a few formulations, containing the selected compounds, for their fungistatic action.

EXPERIMENTAL

Methods of Sterilization.—Tests were conducted to determine whether the active antibacterial compounds could withstand moist heat sterilization without impairment of their bacteriostatic activity.

Nutrient broth containing the previously determined (1) inhibitory concentrations of the test substances were autoclaved at 15 lb. pressure for twenty minutes. Results observed following inoculation of a loopful of a twenty-four hour culture of the test organisms and incubation for twenty-four to forty-eight hours are recorded in Table I. All the bromoanilides were found to lose their activity by this treatment. However, they retained their inhibitory power after being subjected to dry heat sterilization at 150° for one hour.

Penetration Power.—The agar cup-plate method (2) was followed using *M. pyogenes* var. *aureus* and var. *albus* as the test organisms. One cubic cent

* Received February 3, 1958, from the Bombay University Department of Chemical Technology, Bombay 19, India.

This paper is based in part on the theses submitted to the University of Bombay by R. S. Baichwal and Malati R. Baichwal for the degree of Doctor of Philosophy in the Faculty of Technology.

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TABLE I—BACTERIOSTATIC ACTIVITY OF β NAPHTHOL DERIVATIVES AFTER MOIST HEAT STERILIZATION^a

No	Compound	M p. °C	<i>M. pyogenes</i> <i>var aureus</i> Concentration in $\mu\text{g}/\text{cc}$		<i>M. pyogenes</i> <i>var albus</i> Concentration in $\mu\text{g}/\text{cc}$	
			10	5	10	5
1	3 6 Dibromo β -naphthol	127	— ^c	—	—	—
2	1 3 6-Tribromo- β -naphthol	132	—	—	—	+ ^d
3	3 4 6 Tribromo- β naphthol	128	—	—	—	—
4	6 6'-Dibromo β dinaphthol	202	—	—	—	+
5	6- <i>n</i> -Pentyl- β -naphthol	103 5	—	—	—	—
6	6- <i>n</i> -Hexyl- β -naphthol	101 5	—	—	—	—
7 ^b	1-Bromo-2-hydroxy-3-naphthamide	162	+	+	+	+
8 ^b	1 4' Dibromo-2-hydroxy-3-naphthamide	234	+	+	+	+
9 ^b	1 6 Dibromo-2-hydroxy-3-naphthamide	182	+	+	+	+
10 ^b	1 4' 6-Tribromo 2 hydroxy-3 naphthamide	218	+	+	+	+

^a Fifteen lbs pressure for twenty minutes^b Retained bacteriostatic activity after dry sterilization at 150° for one hour^c — no growth ^d + growth

meter of a twenty four hour culture was added to 9 cc of nutrient agar broth and poured into Petri plates containing 20 cc of agar. Fine suspensions of the test compounds in concentrations of 50, 20, and 10 $\mu\text{g}/\text{cc}$ in 5% gum acacia were added to 10 mm cups previously prepared. Diameters of zones of complete inhibition were measured after incubation for forty eight hours. Table II records the zone diameters shown at a concentration of 50 $\mu\text{g}/\text{cc}$ (column 1), and the difference in zone diameters between those at 50 $\mu\text{g}/\text{cc}$ and 10 $\mu\text{g}/\text{cc}$ concentrations (column II).

The penetrability of the more active compounds (Table II), such as 6-*n*-hexyl- β -naphthol, 1 4' dibromo, and 1 6 dibromo-2-hydroxy-3-naphthamides, was evaluated in ointment vehicles using the base recommended for sterilized cream of penicillin, B. P. 1948, by the agar plate method (3). Using *M. pyogenes var aureus*, the zones of complete inhibition compared favorably with those of acriflavine run under similar conditions, and were greater than those exhibited by the parent compound, β -naphthol.

For comparison purposes, antifungal compounds selected from the preliminary screening (1) were also tested under similar conditions (3) using *Trichophyton gypsum* as the test organism. The tests revealed that 8-hydroxyquinoline was the best and the other compounds in decreasing order of activity

were, 1-bromo-, 6-bromo-, 1 6-dinitro β -naphthols, salicylamide, and undecylenic acid.

Influence of Organic Matter.—The bacteriostatic concentrations of the selected compounds (1) were determined using twenty-four hour cultures of *M. pyogenes var aureus* and *var albus* in varying amounts such as 0.02 cc to 0.2 cc (viable count, 382 million/cc). The inhibitory concentrations were found to be the same as determined previously (1) which indicated that the presence of heavy inoculum had no deleterious effect.

Tests using horse serum as the source of organic matter were conducted with the most promising compound, 6-hexylnaphthol, on lines similar to those used for hexachlorophene (4). From the results recorded in Table III, it appears that, although the presence of serum lowers the antibacterial activity, it is only over short durations. However, when long time contact is permitted, then it appears that the presence of serum is not too detrimental. Further, the loss of activity due to presence of serum could be overcome by increasing the concentration.

The antifungal compounds selected from the preliminary screening (1) were evaluated using the serum agar cup plate (2) as well as the agar streak method (5). The former method was similar to that described previously (1) excepting for the addition of 10% serum to the agar medium. From the graphs shown in Fig. 1 of each substance with the log concentration vs. average diameter of the zone of inhibition, comparison of activities can be made. For example, 6-bromo- and 1 6-dinitro β -naphthols seem to be superior to 8-hydroxyquinoline until a concentration of 0.25% is reached. However, when compared with salicylamide or undecylenic acid, the naphthol derivatives are found to show greater zone diameters over the entire range of concentration of 0.01 to 5%.

Following the method of Oster and Golden (6) but using a clearance zone of 3 mm with a cup of 10 mm and allowing a preliminary incubation of the test organism for six hours before the addition of the test substances, as followed in these tests, the activity coefficients calculated are as follows: undecylenic acid 0.4, salicylamide 10, 8-hydroxyquinoline 20, 1-bromonaphthol 40, 6-bromonaphthol 40, and 1 6-dinitronaphthol 100. Apparently, the β -naphthol derivatives seem to be superior to the commonly used antifungal agents under the test conditions followed.

TABLE II—PENETRATION POWER OF β -NAPHTHOL DERIVATIVES AT A CONCENTRATION OF 50 $\mu\text{g}/\text{cc}$

Compound ^a	<i>M. pyogenes</i> <i>var aureus</i>		<i>M. pyogenes</i> <i>var albus</i>	
	Ib	Iic	Ib	Iic
1	14	4	15	4
2	13	2 5	13	3
3	12	2	12	2
4	10	0	10	0
5	10 ^d	0	13 ^d	2 5
6	10 ^d	0	12 ^d	2
7	17 ^d	4	16 ^d	5
8	17	2 5	18	3 5
9	25	8	20	6
10	16	2	21	5

^a Numbers refer to compounds listed in Table I^b Average diameter in mm of the zones of complete inhibition^c Difference between zone diameter at 50 $\mu\text{g}/\text{cc}$ and at 10 $\mu\text{g}/\text{cc}$ ^d Considerable zones of diffused growth were observed

TABLE III.—EFFECT OF ORGANIC MATTER ON THE ANTIBACTERIAL ACTIVITY OF 6-*n*-HEXYL- β -NAPHTHOL

Amount Tested	Results ^a after Contact with <i>M. pyogenes</i> var. <i>aureus</i> for:			
	10 Min.	3 Hr.	24 Hr.	48 Hr.
Hexyl- β -naphthol				
1:1,000 in Serum 50%	...	1,200	0	0
1:1,000 in Serum 10%	1,000	50	0	0
1:1,000 in Serum 0%	100	0	0	0
Hexyl- β -naphthol				
1:2,000 in Serum 50%	2,000	1,500	10	0
1:2,000 in Serum 0%	250	100	0	0

^a Test procedure: mixture allowed to stand at 37° for times noted, after which a standard loopful was transferred to nutrient agar, incubated forty-eight hours, and colonies present counted.
^b Too many to count.

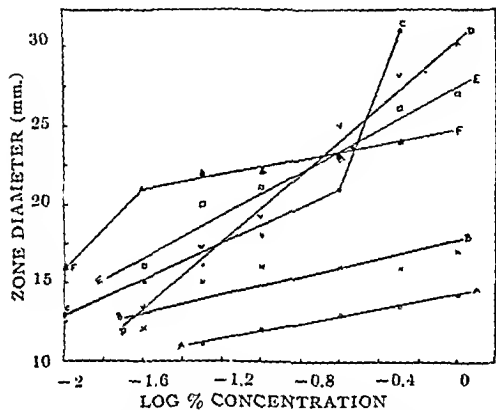


Fig. 1.—Dose and fungistatic response curves of *T. gypsum* with increasing concentrations of β -naphthol derivatives and three commonly used antifungal agents. A-A Undecylenic acid, B-B Salicylanilide, C-C 8-Hydroxyquinoline, D-D 1-Bromo- β -naphthol, E-E 6-Bromo- β -naphthol, and F-F 1:6-Dinitro- β -naphthol.

Results obtained using the agar streak method (5) in which the agar medium contained 10% horse serum, are recorded in Table IV. The activity of 8-hydroxyquinoline against all the fungus pathogens seems to remain unaffected in the presence of serum. With the naphthol derivatives this is true only when their activities are considered against *Trichophyton rubrum* and *Epidermophyton floccosum*. However, these compounds which were inhibitory against the same organisms at a concentration of 10

$\mu\text{g./cc.}$ in the absence of serum (1) were found to be ineffective at the same concentration in the presence of serum.

Fungicidal Tests.—Oster and Golden's method (6) was followed with *T. gypsum* as the test organism and 1% concentration of the test substances. Results after incubation for twenty-one days indicated that 1-bromo- and 1:6-dinitro- β -naphthols were not fungicidal even with a contact period of ten minutes. Other antifungal agents, such as undecylenic acid, salicylanilide or 8-hydroxyquinoline, which were included for comparison purposes, behaved similarly. 6-Bromo- β -naphthol was the only compound which was fungicidal in these tests. Since the compounds were tested at only one concentration, detailed comparisons of activities could not be made.

Irritation Tests.—Ointments containing the test substances in various concentrations were rubbed every day for six days on marked depilated areas on the backs of three healthy rabbits with the ointment base as the control. The ointments were prepared according to the method described under penetration tests. The results indicated that 1:6-dibromo- and 1:4'-dibromo-2-hydroxy-3-naphthanilides were nonirritant even at a concentration of 5%, while 6-hexyl- β -naphthol showed some signs of irritation.

Similar tests conducted with the antifungal agents revealed that undecylenic acid 5%, 8-hydroxyquinoline 1%, 1-bromo- and 6-bromo-naphthols 2%, and 1:6-dinitronaphthol 1% were all nonirritant. Salicylanilide 5%, however, was found to be irritant. Before any definite conclusions are drawn, it would be worth while to test these compounds again on a larger number of animals.

An attempt was next made to study irritation of the bacteriostatic compounds by the "granuloma pouch" test of Hans Selye (7, 8). In the preliminary tests, sterile arachis oil, croton oil, or 6-hexyl- β -naphthol 1% in arachis oil were injected into the prepared pouches (7, 8) on different groups of rats. The animals were sacrificed after seven days, and examination of the pouches indicated that only croton oil was irritant and not the compound or the base oil.

With a view to examining whether the test substances possessed any anti-inflammatory action, the "granuloma pouch" technique (7, 8) was repeated by administering the drugs to different groups of rats, each containing five rats, as shown in Table V. It was observed that the two compounds, 1:6-dibromo-2-hydroxy-3-naphthanilide and 6-hexyl- β -naphthol showed no anti-inflammatory action.

TABLE IV.—FUNGISTATIC ACTIVITY OF β -NAPHTHOL DERIVATIVES AT A CONCENTRATION OF 50 $\mu\text{g./cc.}$ BY AGAR-STREAK METHOD

No.	Compound	<i>Trichophyton gypsum</i>		<i>Trichophyton rubrum</i>		<i>Epidermophyton floccosum</i>	
		Serum Absent	Serum Present	Serum Absent	Serum Present	Serum Absent	Serum Present
1	Undecylenic acid	+	+	+	+	+	+
2	Salicylanilide	+	+	+	+	+	+
3	8-Hydroxyquinoline	—	—	—	—	—	—
4	1-Bromo- β -naphthol	—	+	—	—	—	+
5	6-Bromo- β -naphthol	—	+	—	—	—	—
6	1:6-Dinitro- β -naphthol	—	+	—	—	—	—

^a +, growth. ^b —, no growth.

TABLE V.—ANTI-INFLAMMATORY ACTIVITY OF β -NAPHTHOL DERIVATIVES

Groups	Treatment	Quantity Injected, ml.	Exudate, ml., After Ten Days
I	Arachis oil	0.5	1.2
II	1% Croton oil ^a	0.5	13 \pm 1.5
III	1% Croton oil ^a and 0.5% 6- <i>n</i> -hexyl- β -naphthol ^a	0.5 ^b	13 \pm 1.5
IV	1% Croton oil ^a and 0.5% 1:6-dibromo-2-hydroxy-3-naphthanol ^a	0.5	13 \pm 1.5
		0.5 ^b	...

^a Dissolved in sterile arachis oil.^b Same quantity injected again after three days.

However, they did not seem to contribute any added irritation due to their presence.

Wound Healing Tests.—For this purpose, Carrell and Hartmann's technique (9) used by Dodd and co-workers (10) was followed by using healthy rats instead of rabbits. Three male rats of average body weight of 125 Gm. were depilated, allowed to rest for twenty-four hours, anesthetized, and two excisions made on opposite sides of the dorsal midline. One of the wounds was treated with an ointment containing the test substance and the other with the

base serving as control. The rest of the procedure was similar to that described by Dodd, *et al.* (10). From the results recorded in Table VI, it is apparent that 6-hexyl- β -naphthol and 1:6-dibromo-2-hydroxy-3-naphthanol do not interfere with the process of epithelization in concentrations of 1% and 5%. However, wounds treated with 1:4'-dibromo-2-hydroxy-3-naphthanol, even at 1% concentration require two days more for healing than the control wounds.

In case of antifungal compounds, it was observed that, with all the compounds tested, the days required for the healing of the test and control wounds were the same. Salicylanilide and 6-bromonaphthol which showed some signs of irritation on intact skin of rabbits when applied in the form of 5% ointments, had no adverse effect on the process of wound healing (Table VI).

Toxicity Tests.—An acute toxicity test was carried out with 6-hexyl- β -naphthol using different routes of administration. A stock solution of the substance was prepared using 0.1% Tween 80 and sterilized by autoclaving. Oral administration in doses up to 1 Gm./Kg. showed no toxicity to mice. When administered intravenously, doses up to 40 mg./Kg. were found to have no harmful effects. The LD₅₀ by intraperitoneal route was of the order of 127 mg./Kg.

TABLE VI.—DATA ON WOUNDS TREATED WITH β -NAPHTHOL DERIVATIVES AND CONTROL WOUNDS

Substance Applied	Concn. % in Ointment	2	3	4	5	6	7	8	9	10	11	12
Av. Area in Sq. Cm. of Unhealed Wounds After — days												
6 ^a	1 T	0.96	...	1.03	...	0.86	...	0.55	...	0.23	...	0.06 ^c
	C	1.23	...	1.33	...	1.00	...	0.60	...	0.26	...	0.06 ^c
	5 T	1.10	...	1.20	...	0.76	...	0.50	...	0.50	...	0.23 ^c
	C	1.03	...	1.16	...	0.76	...	0.60	...	0.56	...	0.26 ^c
8 ^a	1 T	1.10	...	0.73	...	0.56	...	0.50	...	0.40	...	0.16 ^c
	C	0.93	...	0.70	...	0.56	...	0.36	...	0.10	...	0
	5 T	1.0	...	0.63	...	0.53	...	0.43	...	0.33	...	0.16 ^c
	C	1.03	...	0.76	...	0.60	...	0.30	...	0.08	...	0
9 ^a	1 T	1.73	...	1.16	...	0.46	...	0.26	...	0.08	...	0
	C	1.60	...	1.16	...	0.50	...	0.23	...	0.08	...	0
	5 T	1.96	...	1.00	...	0.60	...	0.33	...	0.10	...	0
	C	2.00	...	1.30	...	0.63	...	0.43	...	0.20	...	0
1 ^b	5 T	...	0.40	...	0.16	...	0.13	0
	C	...	0.25	...	0.20	...	0.10	0
	2 T	...	0.46	...	0.33	...	0.20	0.03	0	...
	C	...	0.20	...	0.20	...	0.10	0	0	...
2 ^b	5 T	0.83	...	0.6	...	0.50	0.33	...	0.1 ^d	...
	C	0.60	...	0.45	...	0.35	0.20	...	0.1 ^d	...
	2 T	1.00	...	0.73	...	0.66	0.40	...	0.2 ^d	...
	C	0.65	...	0.65	...	0.45	0.15	...	0.05 ^d	...
3 ^b	1 T	...	1.1	...	0.66	0.28	0.13 ^d	...
	C	...	0.6	...	0.5	0.20	0.10 ^d	...
	0.5 T	...	1.0	...	0.75	0.55	0.15 ^d	...
	C
4 ^b	5 T	0.90	...	0.60	...	0.40	0.26	...	0.06 ^d	...
	C	0.60	...	0.45	...	0.35	0.20	...	0.10 ^d	...
	2 T	0.60	...	0.46	...	0.26	0.13	...	0.06 ^d	...
	C	0.65	...	0.65	...	0.45	0.15	...	0.05 ^d	...
5 ^b	5 T	...	1.50	...	1.10	...	0.73	0.26	...	0.20 ^c
	C	...	1.70	...	1.10	...	0.80	0.43	...	0.23 ^c
	2 T	...	0.60	...	0.36	...	0.30	...	0.1	0
	C	...	0.63	...	0.40	...	0.23	...	0.1	0
6 ^b	1 T	...	0.40	...	0.20	...	0.15	0.10	...	0
	C	...	0.30	...	0.20	...	0.15	0.10	...	0
	0.5 T	...	0.20	...	0.20	...	0.10	0	...	0
	C	...	0.20	...	0.20	...	0.10	0.10	...	0

^a Numbers refer to compounds listed in Table I.^b Numbers refer to compounds listed in Table IV.^c Complete healing on fourteenth day.^d Complete healing on thirteenth day.

T Test.

C Control.

Formulations.—In order to determine the synergistic or antagonistic behavior of the selected antifungal compounds, different formulations were prepared, containing a few commonly used antifungal agents. The two most effective antibacterial compounds as shown by these studies were incorporated in some of the mixtures.

The serum agar-cup-plate method described earlier was used with *T. gypsum* as the test organism. For every formulation two mixtures were prepared; one of these (a) contained that concentration of each substance which was able to produce a zone diameter of 16 mm as determined previously (see under influence of organic matter) and the other (b) one-half of those concentrations. Hence if a zone diameter of more than 16 mm was shown by mixture (a), and at least 16 mm by mixture (b), the test substances in those mixtures were considered to be synergistic in their action. Where antibacterial compounds were added in the mixtures, the concentrations used were twenty times their minimum inhibition concentrations against *M. pyogenes* var. *aureus*, determined previously (1).

The results recorded in Table VII indicate that 6-bromo- and 1:6-dinitronaphthols appear to be synergistic with some of the known antifungal agents studied. Similar behavior is shown by 6-hexyl- β -naphthol when combined with 8-hydroxyquinoline. Further detailed studies on these and other combinations are in progress.

SUMMARY

Some derivatives of β -naphthol selected from a previously reported preliminary antibacterial and antifungal screening have been evaluated for their potential value as anti-infective agents. Methods of sterilization, penetration power, influence of organic matter, and fungicidal activity were some of the properties examined. The more effective antibacterial compounds, such as 6-*n*-hexyl- β -naphthol, 1:4', and 1:6-dibromo-2-hydroxy-3-naphthylamides, and the antifungal compounds, 1-bromo-, 6-bromo-, and 1:6-dinitro- β -naphthols, have also been studied for topical irritation and interference in the process of epithelization. Further, the anti-inflammatory activity and the acute toxicity of the bacteriostatic compounds have been determined. Finally, some formulations containing the naphthol derivatives and certain known antifungal agents were examined for any potentiation of fungistatic action.

1-Bromo-, 6-bromo-, 1:6-dinitro-, and 6-*n*-hexyl- β -naphthols and 1:6-dibromo-2-hydroxy-

TABLE VII.—FUNGISTATIC ACTIVITY OF FORMULATIONS CONTAINING β -NAPHTHOL DERIVATIVES AND KNOWN ANTIFUNGAL AGENTS

Formulation	Concn. Respectively, in Final Mixture, %	Av. Zone Diameter, mm I ^a	Zone Diameter, mm II ^b
UDA & SA	2 5, 0 1	14 0	13 0
UDA & 8Q	2 5, 0 05	17 0	15 5
SA & 8Q	0 10, 0 05	19 0	15 0
UDA & 6-Br	2 5, 0 025	15 0	14 5
UDA & Dinitro	2 5, 0 010	15 5	15 0
UDA & 6-Br & Dinitro	2 5, 0 025, 0 010	14 0	14 0
SA & 6-Br	0 1, 0 025	17 0	14 0
SA & Dinitro	0 1, 0 01	17 0	14 0
SA & DiBr	0 1, 0 001	15 0	13 5
8Q & 6-Br	0 05, 0 025	18 0	16 0
8Q & Dinitro	0 05, 0 01	18 0	15 0
8Q & Hexyl	0 05, 0 005	19 0	14 5

^a With concentrations given in column 2

^b With half the concentrations given in column 2

UDA = Undecylenic acid, 6 Br = 6 Bromo β naphthol
Dinitro = 1:6-Dinitro β naphthol DiBr = 1:6-Dibromo 2-hydroxy-3-naphthylamide, Hexyl = 6-*n*-Hexyl β naphthol
SA = Salicylamide, and 8Q = 8 Hydroxyquinoline

3-naphthylamide were found to possess good penetration power in the form of ointments and were nonirritant to the intact skin of rabbits. They did not seem to interfere in the process of healing of wounds nor were their activities seriously affected by organic matter. A synergistic action of some of these compounds was observed in formulations containing certain useful antifungal agents. The LD₅₀ by intraperitoneal administration of 6-hexyl- β -naphthol was of the order of 127 mg./Kg. It appears from these results that the compounds may prove to be useful anti-infective agents and hence merit clinical study.

REFERENCES

- (1) Buchwal, R. S., Buchwal, Malati R., and Khosani, M. L., *This Journal*, **46**, 603 (1957).
- (2) Burlingame, E. M., and Reddish, G. F., *J. Lab. Clin. Med.*, **24**, 771 (1939).
- (3) "Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization," edited by Reddish, G. F., Lea and Febiger, Philadelphia, 1954, p. 91.
- (4) Cade, A. R., and Gump, W. S., "The phenols," in "Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization," edited by Reddish, G. F., Lea and Febiger, Philadelphia, 1954, p. 260.
- (5) Schamberg, J. P., and Kolmer, J. A., *Arch. Dermatol. and Syphilol.*, **6**, 746 (1922).
- (6) Oster, K. A., and Golden, M. J., *This Journal*, **36**, 283, 359 (1957).
- (7) Selye, H., *Proc. Soc. Exptl. Biol. Med.*, **82**, 328 (1953).
- (8) Selye, H., *J. Am. Med. Assoc.*, **152**, 1207 (1953).
- (9) Carrell, A., and Hartmann, A., *J. Exptl. Med.*, **24**, 429 (1916).
- (10) Dodd, M. C., Hartmann, F. W., and Ward, W. C., *This Journal*, **35**, 33 (1916).

TABLE V.—ANTI-INFLAMMATORY ACTIVITY OF β -NAPHTHOL DERIVATIVES

Groups	Treatment	Quantity In- jected, ml.	Exudate, ml., After Ten Days
I	Arachis oil	0.5	1.2
II	1% Croton oil ^a	0.5	13 \pm 1.5
III	1% Croton oil ^a and 0.5% 6- <i>n</i> - hexyl- β -naphthol ^a	0.5 ^b	13 \pm 1.5
IV	1% Croton oil ^a and 0.5% 1:6-di- bromo-2-hydroxy-3- naphthanolide ^a	0.5 ^b	13 \pm 1.5

^a Dissolved in sterile arachis oil.^b Same quantity injected again after three days.

However, they did not seem to contribute any added irritation due to their presence.

Wound Healing Tests.—For this purpose, Carrell and Hartmann's technique (9) used by Dodd and co-workers (10) was followed by using healthy rats instead of rabbits. Three male rats of average body weight of 125 Gm. were depilated, allowed to rest for twenty-four hours, anesthetized, and two excisions made on opposite sides of the dorsal midline. One of the wounds was treated with an ointment containing the test substance and the other with the

base serving as control. The rest of the procedure was similar to that described by Dodd, *et al.* (10). From the results recorded in Table VI, it is apparent that 6-hexyl- β -naphthol and 1:6-dibromo-2-hydroxy-3-naphthanolide do not interfere with the process of epithelization in concentrations of 1% and 5%. However, wounds treated with 1:4'-dibromo-2-hydroxy-3-naphthanolide, even at 1% concentration require two days more for healing than the control wounds.

In case of antifungal compounds, it was observed that, with all the compounds tested, the days required for the healing of the test and control wounds were the same. Salicylanilide and 6-bromonaphthol which showed some signs of irritation on intact skin of rabbits when applied in the form of 5% ointments, had no adverse effect on the process of wound healing (Table VI).

Toxicity Tests.—An acute toxicity test was carried out with 6-hexyl- β -naphthol using different routes of administration. A stock solution of the substance was prepared using 0.1% Tween 80 and sterilized by autoclaving. Oral administration in doses up to 1 Gm./Kg. showed no toxicity to mice. When administered intravenously, doses up to 40 mg./Kg. were found to have no harmful effects. The LD₅₀ by intraperitoneal route was of the order of 127 mg./Kg.

TABLE VI.—DATA ON WOUNDS TREATED WITH β -NAPHTHOL DERIVATIVES AND CONTROL WOUNDS

Substance Applied	Concn. % in Oint- ment	2	3	4	5	6	7	8	9	10	11	12
Av. Area in Sq. Cm. of Unhealed Wounds After — days												
6 ^a	1 T	0.96	...	1.03	...	0.86	...	0.55	...	0.23	...	0.06 ^c
	C	1.23	...	1.33	...	1.00	...	0.60	...	0.26	...	0.06 ^c
	5 T	1.10	...	1.20	...	0.76	...	0.50	...	0.50	...	0.23 ^c
	C	1.03	...	1.16	...	0.76	...	0.60	...	0.56	...	0.26 ^c
8 ^a	1 T	1.10	...	0.73	...	0.56	...	0.50	...	0.40	...	0.16 ^c
	C	0.93	...	0.70	...	0.56	...	0.36	...	0.10	...	0
	5 T	1.0	...	0.63	...	0.53	...	0.43	...	0.33	...	0.16 ^c
	C	1.03	...	0.76	...	0.60	...	0.30	...	0.08	...	0
9 ^a	1 T	1.73	...	1.16	...	0.46	...	0.26	...	0.08	...	0
	C	1.60	...	1.16	...	0.50	...	0.23	...	0.08	...	0
	5 T	1.96	...	1.00	...	0.60	...	0.33	...	0.10	...	0
	C	2.00	...	1.30	...	0.63	...	0.43	...	0.20	...	0
1 ^b	5 T	...	0.40	...	0.16	...	0.13	0
	C	...	0.25	...	0.20	...	0.10	0
	2 T	...	0.46	...	0.33	...	0.20	0.03	0	...
	C	...	0.20	...	0.20	...	0.10	0	0	...
2 ^b	5 T	0.83	...	0.6	...	0.50	0.33	...	0.1 ^d	...
	C	0.60	...	0.45	...	0.35	0.20	...	0.1 ^d	...
	2 T	1.00	...	0.73	...	0.66	0.40	...	0.2 ^d	...
	C	0.65	...	0.65	...	0.45	0.15	...	0.05 ^d	...
3 ^b	1 T	...	1.1	...	0.66	0.28	0.13 ^d	...
	C	...	0.6	...	0.5	0.20	0.10 ^d	...
	0.5 T	...	1.0	...	0.75	0.55	0.15 ^d	...
	C
4 ^b	5 T	0.90	...	0.60	...	0.40	0.26	...	0.06 ^d	...
	C	0.60	...	0.45	...	0.35	0.20	...	0.10 ^d	...
	2 T	0.60	...	0.46	...	0.26	0.13	...	0.06 ^d	...
	C	0.65	...	0.65	...	0.45	0.15	...	0.05 ^d	...
5 ^b	5 T	...	1.50	...	1.10	...	0.73	0.26	...	0.20 ^e
	C	...	1.70	...	1.10	...	0.80	0.43	...	0.23 ^e
	2 T	...	0.60	...	0.36	...	0.30	...	0.1	0
	C	...	0.63	...	0.40	...	0.23	...	0.1	0
6 ^b	1 T	...	0.40	...	0.20	...	0.15	0.10	...	0
	C	...	0.30	...	0.20	...	0.15	0.10	...	0
	0.5 T	...	0.20	...	0.20	...	0.10	0	...	0
	C	...	0.20	...	0.20	...	0.10	0.10	...	0

^a Numbers refer to compounds listed in Table I.^b Numbers refer to compounds listed in Table IV.^c Complete healing on fourteenth day.^d Complete healing on thirteenth day.^e Complete healing on sixteenth day.

T Test.

C Control.

Formulations.—In order to determine the synergistic or antagonistic behavior of the selected antifungal compounds, different formulations were prepared, containing a few commonly used antifungal agents. The two most effective antibacterial compounds as shown by these studies were incorporated in some of the mixtures.

The serum agar-cup-plate method described earlier was used with *T. gypseum* as the test organism. For every formulation two mixtures were prepared; one of these (a) contained that concentration of each substance which was able to produce a zone diameter of 16 mm. as determined previously (see under influence of organic matter) and the other (b) one-half of those concentrations. Hence if a zone diameter of more than 16 mm. was shown by mixture (a), and at least 16 mm. by mixture (b), the test substances in those mixtures were considered to be synergistic in their action. Where antibacterial compounds were added in the mixtures, the concentrations used were twenty times their minimum inhibition concentrations against *M. pyogenes* var. *aureus*, determined previously (1).

The results recorded in Table VII indicate that 6-bromo- and 1:6-dinitronaphthols appear to be synergistic with some of the known antifungal agents studied. Similar behavior is shown by 6-hexyl- β -naphthol when combined with 8-hydroxyquinoline. Further detailed studies on these and other combinations are in progress.

SUMMARY

Some derivatives of β -naphthol selected from a previously reported preliminary antibacterial and antifungal screening have been evaluated for their potential value as anti-infective agents. Methods of sterilization, penetration power, influence of organic matter, and fungicidal activity were some of the properties examined. The more effective antibacterial compounds, such as 6-*n*-hexyl- β -naphthol, 1:4', and 1:6-dibromo-2-hydroxy-3-naphthanilides, and the antifungal compounds, 1-bromo-, 6-bromo-, and 1:6-dinitro- β -naphthols, have also been studied for topical irritation and interference in the process of epithelization. Further, the anti-inflammatory activity and the acute toxicity of the bacteriostatic compounds have been determined. Finally, some formulations containing the naphthol derivatives and certain known antifungal agents were examined for any potentiation of fungistatic action.

1-Bromo-, 6-bromo-, 1:6-dinitro-, and 6-*n*-hexyl- β -naphthols and 1:6-dibromo-2-hydroxy-

TABLE VII.—FUNGISTATIC ACTIVITY OF FORMULATIONS CONTAINING β -NAPHTHOL DERIVATIVES AND KNOWN ANTIFUNGAL AGENTS

Formulation	Concn., Respectively, in Final Mixture, %	Av. Zone Diameter, mm.	
		I ^a	II ^b
UDA & SA	2.5, 0.1	14.0	13.0
UDA & 8Q	2.5, 0.05	17.0	15.5
SA & 8Q	0.10, 0.05	19.0	15.0
UDA & 6-Br	2.5, 0.025	15.0	14.5
UDA & Dinitro	2.5, 0.010	15.5	15.0
UDA & 6-Br & Dinitro	2.5, 0.025, 0.010	14.0	14.0
SA & 6-Br	0.1, 0.025	17.0	14.0
SA & Dinitro	0.1, 0.01	17.0	14.0
SA & DiBr	0.1, 0.001	15.0	13.5
8Q & 6-Br	0.05, 0.025	18.0	16.0
8Q & Dinitro	0.05, 0.01	18.0	15.0
8Q & Hexyl	0.05, 0.005	19.0	14.5

^a With concentrations given in column 2.

^b With half the concentrations given in column 2.

UDA = Undecylenic acid; 6-Br = 6-Bromo- β -naphthol; Dinitro = 1:6-Dinitro- β -naphthol; DiBr = 1:6-Dibromo-2-hydroxy-3-naphthanilide; Hexyl = 6-*n*-Hexyl- β -naphthol; SA = Salicylanilide; and 8Q = 8-Hydroxyquinoline.

3-naphthanilide were found to possess good penetration power in the form of ointments and were nonirritant to the intact skin of rabbits. They did not seem to interfere in the process of healing of wounds nor were their activities seriously affected by organic matter. A synergistic action of some of these compounds was observed in formulations containing certain useful antifungal agents. The LD₅₀ by intraperitoneal administration of 6-hexyl- β -naphthol was of the order of 127 mg./Kg. It appears from these results that the compounds may prove to be useful anti-infective agents and hence merit clinical study.

REFERENCES

- (1) Baichwal, R. S., Baichwal, Malati R., and Khorana, M. L., *THIS JOURNAL*, 46, 603(1957).
- (2) Burlingame, E. M., and Reddish, G. F., *J. Lab. Clin. Med.*, 24, 771(1939).
- (3) "Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization," edited by Reddish, G. F., Lea and Febiger, Philadelphia, 1954, p. 91.
- (4) Cade, A. R., and Gump, W. S., "The bis-phenols," in "Antiseptics, Disinfectants, Fungicides, and Chemical and Physical Sterilization," edited by Reddish, G. F., Lea and Febiger, Philadelphia, 1954, p. 250.
- (5) Schamberg, J. F., and Kolmer, J. A., *Arch. Dermatol. and Syphilol.*, 6, 746(1922).
- (6) Oster, K. A., and Golden, M. J., *THIS JOURNAL*, 36, 283, 359(1917).
- (7) Selye, H., *Proc. Soc. Exptl. Biol. Med.*, 82, 328(1953).
- (8) Selye, H., *J. Am. Med. Assoc.*, 152, 1307(1953).
- (9) Carrell, A., and Hartmann, A., *J. Exptl. Med.*, 24, 429(1916).
- (10) Dodd, M. C., Hartmann, F. W., and Ward, W. C., *THIS JOURNAL*, 35, 33(1916).

The Excretion and Tissue Distribution of C¹⁴-Carbonyl Salicylamide in the Rat*

By WILLIAM F. BOUSQUET and JOHN E. CHRISTIAN

This investigation was undertaken to determine the excretion and tissue distribution of C¹⁴-carbonyl labeled salicylamide and its metabolites in the rat. Twenty-four hours following the administration of 250 mg./Kg. of salicylamide containing 2.35 μ c. of C¹⁴, 96.9 per cent of the administered radioactivity was found in the urine. Thirty minutes following the oral administration of 250 mg./Kg. of the compound containing 4.35 μ c. of C¹⁴ the greatest quantity of the drug and its metabolites was found in the kidney. The brain-plasma ratio expressed on a dry weight basis was found to be 1.72.

THE ANALGETIC VALUE of salicylamide is well recognized (1, 2) and has been reported by some workers to be greater than that of aspirin (3). It appears that salicylamide differs greatly from other members of the salicylate group of drugs in its metabolism and mode of action. The findings of Brodie and Szekely (4) would indicate that the compound is not converted to salicylate in the animal body.

Salicylamide seems to be free of the untoward effects often noted with aspirin therapy (5, 6). The compound appears to be well absorbed and no gastric ulceration has been noted upon prolonged administration. Changes in red and white cell counts or in prothrombin time have not been reported to occur with salicylamide therapy (6).

The overall excretory pattern of salicylamide was studied by administering the drug labeled with C¹⁴ to rats, collecting the urine and feces separately over a period of twenty-four hours, and analyzing the excretion products for their radioactive content.

To determine the tissue distribution of the drug and/or its metabolites various tissues and organs were taken for assay thirty minutes following the oral administration of the compound labeled with C¹⁴. These organs and tissues were then brought into a suitable form for analysis and their radioactive content determined.

EXPERIMENTAL

Synthesis of C¹⁴-Carbonyl Salicylamide.—The labeled compound used in this study was prepared using C¹⁴-carboxyl salicylic acid previously synthesized in this laboratory by Borst and Christian (7). The procedure reported by Mandel, *et al* (8), was modified for use in this work. The synthesis was carried out by refluxing 0.5648 Gm. of C¹⁴ carboxyl

salicylic acid representing 1.51×10^8 d.p.m. of radioactivity with 80 ml. of absolute methanol for twenty-four hours in the presence of one drop of concentrated sulfuric acid. When the refluxing period was complete 10 ml. of cooled 28% ammonia solution was added to the reaction mixture and the flask shaken mechanically for eighteen hours.

The reaction mixture was then cooled in an ice bath and the salicylamide and unreacted salicylic acid precipitated by addition of cold 20% hydrochloric acid added dropwise. After refrigeration for two days during which time cold 20% hydrochloric acid was added intermittently to insure completeness of precipitation, the mixture was suction filtered and the salicylamide and unreacted salicylic acid collected. The filtrate and washings were then concentrated, cooled, and allowed to crystallize. This procedure was repeated three times. All solid material obtained in the crystallizations was combined and this material was then treated with a saturated solution of sodium carbonate to remove any free salicylic acid present. Total yield of C¹⁴ carbonyl salicylamide was 0.4039 Gm. or 72% of theory before recrystallization. Recrystallization from water yielded 0.2960 Gm. of material having a melting point of 140° (M.M.P.-K.) which is in agreement with the literature value for salicylamide (9). Total yield of purified product was 52% of theory.

Demonstration of Chemical and Radiochemical Purity.—Purity of the labeled compound was ascertained by subjecting it to chromatographic and autoradiographic analysis. An acetone solution of the synthesized C¹⁴-carbonyl salicylamide was prepared in a concentration of 30 gamma per 5 lambda. A 5 lambda sample of this solution was chromatographed on Whatman No. 1 paper using the descending method of paper partition chromatography. The solvent system employed was benzene-glacial acetic acid-water (40:40:20). Reference samples of unlabeled salicylic acid and salicylamide were run as controls on the same paper. The *R_f* value of the synthesized C¹⁴-carbonyl salicylamide was determined to be 1.0, indicating that the labeled compound was identical with the salicylamide reference compound. No salicylic acid could be demonstrated to be present in the synthesized C¹⁴ carbonyl salicylamide upon color development of the chromatogram with a 1% solution of ferric chloride. To demonstrate the absence of radiochemical impurities in the synthesized compound an autoradiogram of the chromatogram was prepared. Eastman

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Kodak "No-Screen" X-ray film was used in preparing the autoradiogram and the exposure time was calculated on the basis of the known activity present on the chromatogram. Upon film development there was present but one spot showing that there were no radiochemical impurities present in the synthesized product.

Activity Determination.—Samples of the synthesized C^{14} -carbonyl salicylamide were dissolved in acetone for determination of their specific activities. The activity¹ of aliquots of the synthesized C^{14} -carbonyl salicylamide showed that the product had a specific activity of 1.18 μ c./mg.

Studies of Salicylamide Excretion.—To each of five white female rats of the Purdue-Wistar strain (average weight of each was 202 Gm.) was administered 250 mg./Kg. of salicylamide containing 2.35 μ c. of C^{14} . Sufficient labeled material to give the desired activity was ground in a glass mortar with unlabeled salicylamide, wetted with a few drops of Aerosol OT,² and suspended with the aid of a 0.5% solution of Kelgin XL.³ The suspension was administered to the animals by means of a stomach tube.

Upon administration of the drug each animal was placed in a metabolism chamber similar to that described by Roth, *et al.* (10), which is equipped to mechanically separate the urine and the feces. For sixteen hours prior to determining salicylamide excretion and during the entire twenty-four hour period of study the animals were allowed water freely but food was withheld. The animal was held in the chamber and the urine and feces collected over a twenty-four hour period.

The urine and feces samples from the five animals were pooled and the aliquot assayed taken as a representative sample of the excretory products of the five animals. The feces sample was homogenized with 10% nitric acid to provide uniform sampling. Samples of the excretion products were then deposited on aluminum counting planchets of 7.31 cm.² area, and the radioactivity of the samples determined with the counting set up as previously described.

Upon assay of the excretory samples it was found that 96.9% of the administered radioactivity was excreted in the urine and that 0.61% was found in the feces. Clearly, then, the kidney represents the organ of excretion of this drug.

Determination of the Tissue Distribution of C^{14} -Carbonyl Salicylamide and/or Its Metabolites.—In order to determine the distribution in the animal body of salicylamide and/or its metabolites a dose of salicylamide corresponding to 250 μ g./Kg. and containing 4.35 μ c. of C^{14} was administered to five white female rats of the Purdue-Wistar strain, the average weight of which was 211 Gm. Prior to administration of the drug the animals were fasted for twenty-two hours but were allowed water freely.

The drug was prepared and administered as previously described. Thirty minutes later the animals were sacrificed by decapitation, the blood being collected and pooled in a graduated centrifuge tube containing ammonium oxalate solution. The blood was centrifuged at 3,000 r. p. m. for thirty minutes and the clear plasma removed by means of a pipet. To the red blood cells remaining was added an equal volume of distilled water in which the cells were allowed to hemolyze for ten hours.

The heart, lung, spleen, liver, adrenals, brain, and kidneys of the animals were removed, pooled, and homogenates prepared in 10% nitric acid with the aid of a tissue grinder. The homogenates were prepared such that a 0.250-ml. sample would contain approximately 10 mg. of dry weight of tissue. Tissue and blood samples were prepared in duplicate for determination of their specific activities. All samples were corrected for self absorption, back-ground, and efficiency of the counting set up. Counting was carried out sufficiently long to provide results within a maximum error of 5%. Table I presents the data obtained. The figures given are a numerical average of duplicate determinations.

TABLE I.—TISSUE DISTRIBUTION OF SALICYLAMIDE AND ITS METABOLITES THIRTY MINUTES AFTER THE ORAL ADMINISTRATION OF C^{14} -CARBONYL SALICYLAMIDE TO THE RAT

Sample	Activity, d. p. m./ mg., Wet Wt.	Activity, d. p. m./ mg., Dry Wt.	Tissue- Plasma Ratio, Wet Wt. Basis	Tissue- plasma Ratio, Dry Wt. Basis
Plasma	32	532	1.00	1.00
R. B. C.	...	195	...	0.36
Kidney	296	1,435	9.20	2.70
Brain	218	917	6.50	1.72
Heart	168	672	5.25	1.36
Lung	53	271	1.65	0.51
Spleen	118	503	3.70	0.94
Liver	73	306	2.25	0.57
Adrenal	29	141	0.90	0.26

DISCUSSION

From the results obtained in the determination of the excretion of salicylamide and its metabolites it is clear that urinary excretion plays the major role in this species. The low level of activity found in the feces is undoubtedly due to contamination by urine and does not indicate excretion of the drug by this route.

It appears significant that salicylamide and/or its metabolites are found in brain tissue in relatively higher concentration than in other tissues with the exception of the kidney. This accumulation warrants further studies as a possible explanation of the higher level of analgetic activity of salicylamide as opposed to others of the salicylate group of drugs reported by several authors (1-3).

SUMMARY

The synthesis of C^{14} -carbonyl salicylamide from C^{14} -carboxyl salicylic acid by a modification of the procedure of Mandel, *et al.* (8), has been

¹ All radioactivity determinations in this work were made utilizing a Nuclear Instrument and Chemical Corp. end window tube of 1.4 mg./cm.² thickness in combination with a Radiation Instrument Development Laboratories Model 512 decade scaling unit. The tube was shielded with lead, the background ranging from 12-18 c. p. m. The efficiency of this counting set up for C^{14} was determined as being 2.55% utilizing a National Bureau of Standards C^{14} Sodium Carbonate Standard.

² American Cyanamid Co.

³ Sodium Alginate, Low Viscosity, Kelco Co., N. Y.

described. Identification and radiochemical purity have been established by a combination of chromatography and autoradiography.

A study of salicylamide excretion during a twenty-four hour period following the oral administration of 250 mg/Kg. of salicylamide containing 2 35 μ c of C^{14} in the presence of a non-ionic surfactant revealed that 96.9 per cent of the administered dose was excreted in the urine and that 0.61 per cent was found in the feces.

The tissue distribution of salicylamide and its metabolites in the rat thirty minutes after the oral administration of 250 mg/Kg. of salicylamide containing 4 35 μ c. of C^{14} in each dose and in the presence of a nonionic surfactant has been determined for the lung, heart, kidney, brain, liver, spleen, adrenals, plasma, and the red blood cells. The greatest accumulation of the drug and/or its metabolites was found in the kidney.

The relatively high concentration of the drug in the brain tissue is indicative of the ability of this drug to penetrate the blood-brain barrier to a greater extent than has been reported for the salicylates.

REFERENCES

- (1) Hart, E R, *J. Pharmacol Exptl Therap*, 89, 203 (1947).
- (2) Brodie, D C, Way, E L, and Smith, G, *THIS JOURNAL*, 41, 48(1952).
- (3) Gibson, R D, Miya, T. S, and Edwards, L D, *ibid*, 44, 605(1955).
- (4) Brodie, D C, and Szekely, I J, *ibid*, 40, 414(1951).
- (5) Ichniowski, C T, and Hueper, W. C, *ibid*, 35, 223 (1946).
- (6) Bavin, E M, Macrae, F J, Seymour, D E, and Waterhouse, P D, *J Pharm and Pharmacol*, 4, 872(1952).
- (7) Borst, W R, and Christian, J E, *THIS JOURNAL*, 45, 23(1956).
- (8) Mandel, H G, Rodwell, V W, and Smith, P K, *J Pharmacol Exptl Therap*, 106, 433(1952).
- (9) Hodgman, C D, "Handbook of Chemistry and Physics," 37th ed, Chemical Rubber Publishing Co, Cleveland 1956.
- (10) Roth, L J, Leifer, E, Hogness, J. R, and Langham, W H, *J Biol Chem*, 176, 249(1948).

A Histological Study of Some Mucilaginous Seeds*

By MAHMOUD D. SAYED† and JACK L. BEAL

A description of the morphology and histology of the seeds of Kentucky coffee-tree, thornless honey locust, and sweet basil is given. Mucilage is located in the endosperm of the first two seeds, and in the epidermis of the seed coat of the third.

THE ECONOMIC IMPORTANCE as well as the pharmaceutical and therapeutic importance of certain types of mucilaginous seeds is a well-established fact. This paper concerns a histological study of mucilaginous seeds of three different plants. They are *Gymnocladus dioica* (L.) Koch, kentucky coffee-tree, *Gleditsia triacanthos* L var *inermis*, thornless honey locust, and *Ocimum basilicum* L, sweet basil. The seeds of these plants are of potential commercial importance since seeds of the first two possess a mucilage similar to guar mucilage while the third is more of the order of plantago seeds. Current research is being carried out at this institution on the physical properties and the chemistry of the mucilage of these seeds. It is anticipated that the results will be published at a later date.

KENTUCKY COFFEE-TREE

Gymnocladus dioica (L.) Koch of the *Leguminosae*

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family, commonly known as the kentucky coffee-tree, is a tree 20 to 25 meters in height. It has bipinnate leaves; the leaflets being ovate or oval and range from 2.0 to 7.0 cm long. The flowers are terminal racemes and are described as polygamous, regular. The fruits are pods, being flat and oblong with a very hard texture (1).

Morphology of the Seed.—Kentucky coffee beans, Fig 1, are very hard button-shaped seeds varying from 1.5 to 2.5 cm in length, and 0.3 to 0.5 cm in thickness. The seed coat is smooth and dark brown in color. On the circumference, there is an oval depression marking the position of micropyle and hilum. The seed is albuminous. The endosperm is mucilaginous and swells enormously when brought in contact with water.

A longitudinal section parallel to the flat surface, Fig. 1,¹ shows a large circular cotyledon, filling most of the concavity of the seed, and a small conical radicle directed toward the micropyle. The embryo is surrounded by a narrow translucent mucilaginous endosperm.

Histology.—The seed coat consists of an epidermis, a hypodermis, an intermediate (nutrient) layer, and an innermost collapsed layer.

The epidermis, Fig. 2, consists of palisade-like

¹ The pictures in this paper were photostated by the Photography Department, The Ohio State University

cells varying from 18.5 to 23.0μ in height and from 0.9 to 1.2μ in thickness. They have thick cellulosic walls and are covered with a thick cuticle. In surface view, Fig. 3, the epidermal cells appear poly-

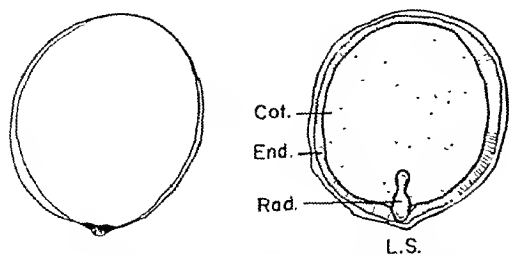


Fig. 1.—Kentucky coffee. $\times 3$. Longitudinal section (L. S.); cotyledon (Cot.); endosperm (End.); radicle (Rad.). $\times 2$.

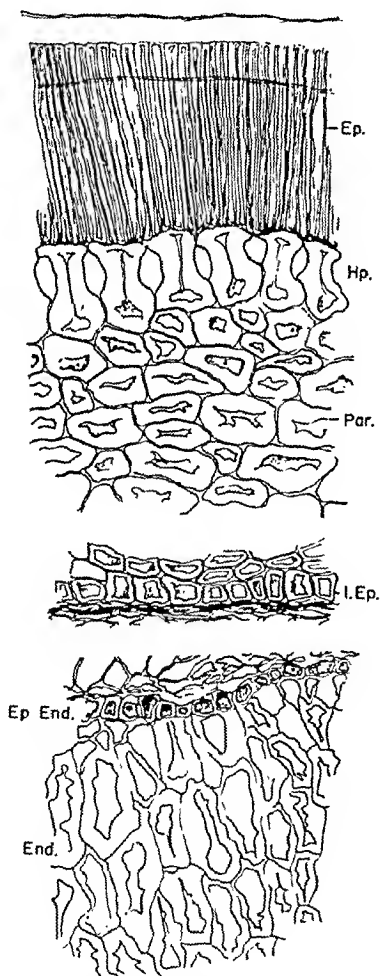


Fig. 2.—Transverse section of Kentucky coffee seed. Epidermis (Ep.); hypodermis (Hp.); intermediate parenchyma (Par.); inner epidermis of seed coat (I. Ep.); epidermis of endosperm (Ep. End.); endosperm (End.). $\times 145$.

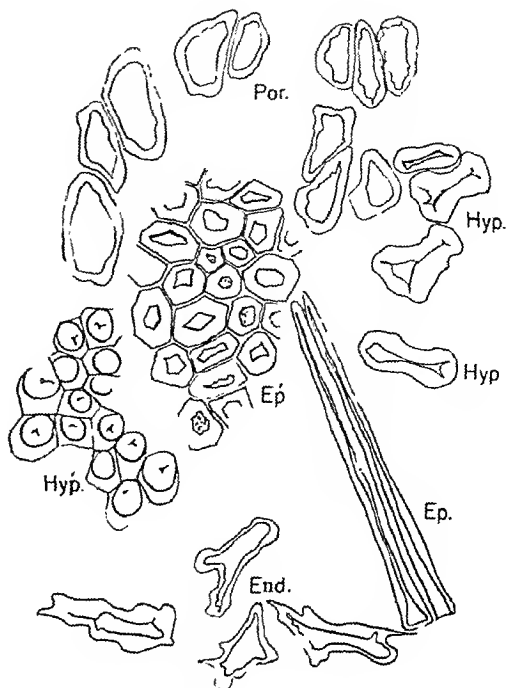


Fig. 3.—Isolated elements of Kentucky coffee seed. Epidermis of seed coat in lateral view (Ep.); same in surface view (Ep.); intermediate parenchyma (Par.); endosperm cells (End.); hypodermis in lateral view (Hyp.); same in surface view (Hyp.). $\times 148$.

gonal in outline with circular lumina. The epidermal tissue was marked by a horizontal line which appeared approximately 6.5μ from the apex. This was of some concern for it gave the appearance of the tissue being made up of two rows of cells; a long row and a short row joined end to end, rather than one row. It was proved to be one row by measuring the length of the cells while intact in the tissue and then measuring them after being separated from the tissue by maceration. Their length within the tissue and after separation was found to be the same indicating that the epidermal tissue consists of one row of cells having the appearance of two rows. In the study of the isolated cells, the appearance of this line was not so distinct as in the transverse section. It is probably due to a difference in the refractive index of the cell wall in the particular area. This phenomenon is to be studied further at this institution.

The hypodermis, Fig. 2, consists of one layer of beaker cells having the shape of short-necked bottles, with cellulosic-thickened walls and narrow lumina which widen at both extremities of the cells assuming irregular triangular shapes. Between these cells there are wide oval intercellular spaces. These cells vary from 4.9 to 5.6μ in height and 1.7 to 2.3μ in breadth (at the broadest part), and 0.6 to 0.9μ at the narrowest part (neck). In surface view, Fig. 3, they appear circular or oval in outline with stellate lumina.

The intermediate parenchymatous tissue (nutrient layer) consists of 9 to 12 layers of isodiametric

or tangentially elongated cells with thick cellulosic walls and narrow triangular intercellular spaces. These cells contain amorphous granular contents reacting for protein by staining yellow with picric acid and brown with iodine test solution.

The inner epidermis of the seed coat, Fig. 2, consists of one row of rectangular cells. This is followed by a layer of collapsed parenchymatous cells which are colored blue by iodine-sulfuric acid indicating their cellulosic nature.

The epidermis of the endosperm, Fig. 2, consists of polygonal cells containing numerous minute aleurone grains that are free of any inclusion, i. e., devoid of crystalloids and globoids. The remainder of the endosperm is formed of axially elongated cells with thick cellulosic walls and irregular lumina.

The following additional microchemical tests were applied on a series of sections of the seed:

1. The walls of the endosperm cells, other than those of the epidermis, swelled enormously when brought in contact with water.
2. Iodine test solution stained the contents of the epidermis of the endosperm as well as the cells of the cotyledons yellowish-brown.
3. None of the tissue stained blue with iodine test solution.
4. Ruthenium red failed to stain the mucilage of the endosperm.
5. Methylene blue stained the contents of the endosperm cells blue.
6. Fuchsin in absolute alcohol stained the contents of the endosperm red.
7. The contents of the embryo stained red with Sudan III test solution.
8. Chlorzinciodine stained the cell walls of the endosperm and embryo violet.
9. The contents of the endosperm and embryo cells stained yellow to yellowish-brown.
10. The cell walls did not give a positive lignin test.

From the above tests one may conclude the following:

1. The cell walls were nonlignified.
2. The epidermis of the endosperm contains aleurone grains while the remainder of the cells of the endosperm contain mucilage.
3. The cells of the embryo contain fixed oils and aleurone grains.
4. Starch is absent in the cells of all the tissues.

THORNLESS HONEY LOCUST

Gleditsia triacanthos L. var. *inermis* of the *Leguminosae* family is commonly known as the thornless honey locust. The plant is a tree with bipinnate leaves, leaflets often crenulate. The flowers are small and are in axillary or lateral racemes or panicles. The fruit is a pod, linear, 20 to 30 cm. in length, curved, and twisted (2).

Morphology of the Seeds.—The seeds, Fig. 4, are oval varying from 0.5 to 1.0 cm. in length, 4.0 to 7.5 mm. in breadth, and 20 to 50 mm. in thickness. The seed coat is smooth and tan to brown in color. At one end of the seed there is a small oval depression marking the position of the embryo. The seed is albuminous. The endosperm is mucilaginous and swells with water and aqueous reagents. A longitudinal section of the seed, Fig. 4, shows a large flat cotyledon and a small radicle surrounded by a mucilaginous endosperm.

Histology.—The seeds of the thornless honey locust show the same arrangement and type of tissues as the seeds of the Kentucky coffee-tree. The following are the important diagnostic features of the powder (Fig. 5):

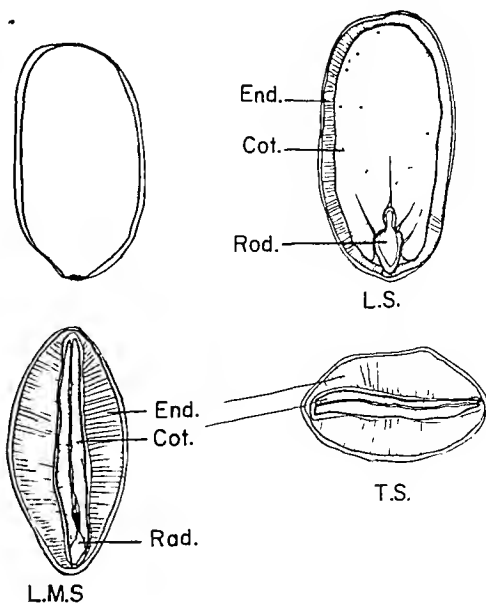


Fig. 4.—Honey locust seed. Longitudinal section parallel to the flat surface (L. S.); cotyledon (Cot.); endosperm (End.); radicle (Rad.); longitudinal median section (L. M. S.); transverse cut surface (T. S.). $\times 4.6$.

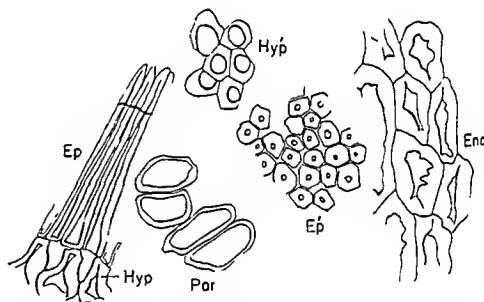


Fig. 5.—Isolated elements of honey locust seed. Epidermis of the seed coat in lateral view (Ep.); same in surface view (Ep.); hypodermis in lateral view (Hyp.); same in surface view (Hyp.); parenchyma of intermediate layer (Par.); endosperm (End.). $\times 110$.

1. Cells of the epidermis are palisade-like and are 13.2 to 16.5 μ in length and 0.6 to 0.9 μ in breadth.
2. Hypodermal cells are basket shaped with cellulosic thickened radial walls.
3. Parenchymatous cells of the intermediate layer had cellulosic walls and were tangentially elongated.
4. Endosperm cells consisting of axially elongated cells with irregular lumina and mucilaginous contents.

SWEET BASIL

Ocimum basilicum L. of the *Labiatae* family, commonly known as sweet basil, is an annual plant much branched 30 to 60 cm. in height. The leaves are ovate, petiolate, 2.5 to 5.0 cm. in length, with

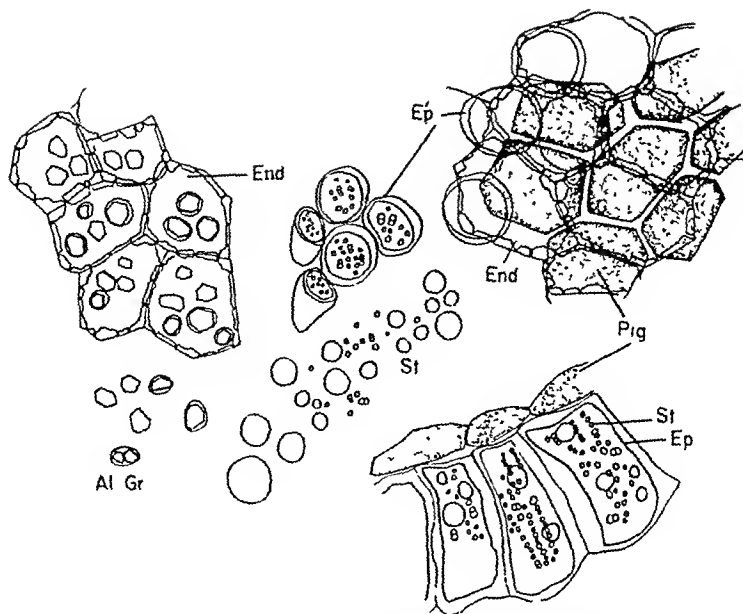


Fig 7.—Isolated elements of sweet basil seed. Epidermis of seed coat in lateral view (Ep) same in surface view (Ep); starch grains (St.); pigment layer (Pig); endosperm (End); aleurone grains (Al. Gr.) $\times 110$.

an entire or toothed margin. The flowers are white, or more or less tinged purple, in moderately dense racemes. *Basilicum* in Greek means kingly because of its healing property. It is native to tropical Asia and the Pacific islands (2).

Morphology of the Seeds.—The seeds are hard in texture, ovoid in shape, varying from 2.0 to 3.0 mm in length and from 0.5 to 1.0 mm in breadth. The seed coat is dark brown in color showing a minutely pitted surface when viewed with a magnifying lens. The seed is albuminous with an oily endosperm surrounding the minute embryo.

Histology.—The seed coat consists of an epidermis, a pigment layer, and an inner layer of large parenchymatous cells. The epidermis, Fig 6, consists of large axially elongated cells varying from 13 to 19.5 μ in height and 6.6 to 11.5 μ in breadth. Their walls are cellulose in nature. The radial walls are more thickened than the outer and inner tangential walls. These cells swell enormously when brought in contact with water and their walls stain blue to purplish blue with methylene blue indicating that they are impregnated with mucilage. The lumina of the cells are wide and contain numerous minute simple and a few 2- to 4-compound starch grains. In surface view, Fig 7, these cells appear rounded with circular lumina. Underneath the epidermis there is one and occasionally two rows of tubular cells containing a dark brown content which is responsible for the color of the seed coat. In surface view, Fig 7, they appear polygonal in outline usually adhering to the cells of the epidermis and endosperm. The innermost layer of the seed coat consists of one row of large parenchymatous cells with thin cellulose walls. The storage tissue in these seeds consists of the endosperm and the two cotyledons.

The endosperm, Fig 6, consists of isodiametric

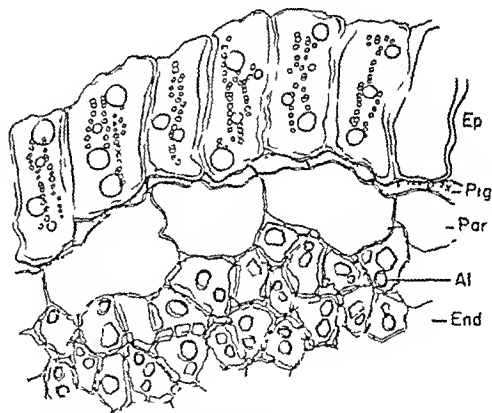


Fig 6. Transverse section in sweet basil seed. Epidermis (Ep); pigment layer (Pig); parenchymatous layer (Par.); aleurone grains (Al.); endosperm (End), $\times 148$.

cells. They have thick-beaded walls with distinct simple pits. Their contents stain red with Sudan III and yellowish-brown with iodine indicating the presence of oil and protein respectively. The protein is present in the form of oval aleurone grains, containing one and occasionally two crystalloids but no globoids.

The embryo cells contain numerous aleurone grains and oil globules.

REFERENCES

- (1) Rydberg, P. A., "Flora of the Prairies and Plains of Central North America," The New York Botanical Garden, 1932, p. 449.
- (2) Bailey, L. H., "Manual of Cultivated Plants," Macmillan Company, New York, 1919, p. 863.

Nonaqueous Titration of the Tetracycline Antibiotics and Some Commercial Preparations*

By F. YOKOYAMA and L. G. CHATTEN

The application of visual nonaqueous titrimetry has been made to the assay of the tetracycline antibiotics and their commercial preparations. Determination is carried out in nitromethane-formic acid-benzene system using mixed indicator comprised of methylene blue and quinaldine red.

THE WIDE USE and the therapeutic importance of the tetracycline antibiotics has resulted in an increased number of pharmaceutical forms of these compounds. The necessity of carefully maintained quality control has prompted numerous investigations into analytical techniques in an effort to cope with the problems which have arisen. In addition to the microbiological determination using the turbidimetric and cylinder-plate methods a considerable number of fluorometric and colorimetric procedures have been proposed (1-12). On the other hand, very little has been published on the application of nonaqueous titrimetry as a chemical means of analysis of these preparations. Sideri and Osol (13) as well as Ekeblad (14) have reported the determination of some of the tetracyclines in glacial acetic acid, but we have been unsuccessful in reproducing satisfactory results by their method. The microbiological technique is still the most widely used, although many experts agree that the recoveries may deviate as much as plus or minus ten per cent from the mean value.

The purpose of this study is to investigate the application of nonaqueous procedures which can be readily performed by visual titration for both the free bases and their hydrohalide salts. The work has been extended to include tetracycline, chlortetracycline, and oxytetracycline compounds.

EXPERIMENTAL

Apparatus.—Five-cubic centimeter buret, graduated in 0.01 cc. or 10-cc. buret, graduated in 0.05 cc.; electromagnetic stirrer; Fisher titrimer, (Model No. 9-311A); silver-silver chloride electrode, (Beckman No. 1264); and glass electrode (Fisher No. 9-312-25).

* Received December 27, 1957, from the Pharmaceutical Chemistry Section, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada.

The authors express their appreciation to Dr. J. E. Logan, Miss K. Fitzpatrick, and B. W. Griffiths of the Laboratory of Hygiene, Department of National Health and Welfare, for carrying out the microbiological and spectrophotometric assays. They would also like to extend their thanks and appreciation to Lederle Laboratories Division, North American Cyanamid Limited and Pfizer Canada for the generous samples of the pure antibiotics.

Reagents.—Nitromethane, practical grade, obtained from Eastman Kodak (redistilled, fraction collected between 100 and 101°); benzene, A. C. S.; formic acid, 85 to 90%, A. C. S.; perchloric acid, 0.05 *N* in dioxane, standardized against potassium acid phthalate, A. C. S.; mixed indicator comprised of 0.1 Gm. methylene blue and 0.2 Gm. quinaldine red in 100 cc. anhydrous methanol (methyl hydrate, absolute B. D. H.); and mercuric acetate, C. P., 6% in glacial acetic acid, A. C. S.

PROCEDURES

Pure Antibiotics.—Accurately weigh a sample of about 50 mg. into a 150-cc. beaker, dissolve in 50 cc. of nitromethane containing 1 cc. of formic acid by stirring electromagnetically. Add 5 cc. of benzene, 1 cc. of 6% mercuric acetate in glacial acetic acid where a hydrochloride salt is to be analyzed, and 0.1 cc. of the mixed indicator. Titrate with 0.05 *N* perchloric acid in dioxane to the end point indicated by the appearance of the green color. Each cc. of 0.05 *N* perchloric acid is equivalent to: 25.77 mg. chlortetracycline (Aureomycin®, Lederle) hydrochloride, 24.85 mg. oxytetracycline (Terramycin®, Pfizer) hydrochloride, 23.02 mg. oxytetracycline (Terramycin®, Pfizer) base, 24.05 mg. tetracycline (Achromycin®, Lederle) hydrochloride, and 22.22 mg. tetracycline (Achromycin®, Lederle) base.

Commercial Preparations.—Capsules and Tablets.—Weigh and powder 20 tablets or weigh the contents of 20 capsules. Place an accurately weighed sample equivalent to 50 mg. of the drug in a 150-cc. beaker. Add 25 cc. of nitromethane containing 1 cc. of formic acid and stir electromagnetically for thirty minutes. Filter through a Whatman No. 1 filter paper into a 150-cc. beaker. Wash the residue with an additional 25 cc. of nitromethane. Add 5 cc. of benzene, 1 cc. of 6% mercuric acetate in glacial acetic acid, and 0.1 cc. of the mixed indicator. Titrate to a green end point with 0.05 *N* perchloric acid in dioxane. Where the formulation includes magnesium stearate, titrate to a blue end point before the addition of mercuric acetate, then add mercuric acetate and continue titration to a green end point.

Ointments.—(a) Chlortetracycline (Aureomycin) hydrochloride and Tetracycline (Achromycin) hydrochloride ointments.

Accurately weigh a sample equivalent to 50 mg. of drug in a 150-cc. beaker, add 2 cc. of formic acid, 50 cc. of nitromethane, 40 cc. of benzene and warm on a hot water bath until the ointment is uniformly dispersed. Add an additional 10 cc. of benzene, 1 cc. of 6% mercuric acetate in glacial acetic acid, and 0.1 cc. of the mixed indicator. Titrate while hot to a green end point with 0.05 *N* perchloric acid in dioxane.

(b) Oxytetracycline (Terramycin) hydrochloride ointment with polymyxin B sulfate.

Accurately weigh a sample equivalent to 50 mg. of

drug in a 150-cc. beaker, add 50 cc. of benzene and warm on a hot water bath until the ointment is uniformly dispersed. Stir electromagnetically while cooling. Filter through a medium porosity sintered glass funnel and extract the residue with 50 cc. of nitromethane by filling the funnel. Omit suction, allowing the filtration to proceed under the influence of gravity. Add 5 cc. of benzene, 1 cc. of formic acid, 1 cc. of 6% mercuric acetate in glacial acetic acid, and 0.1 cc. of the mixed indicator and titrate to a green color with 0.05 *N* perchloric acid.

Oxytetracycline (Terramycin) Hydrochloride Intravenous Injection.—Completely wash out the content of a single ampul labeled to contain 250 mg. of active constituent with 50 cc. of nitromethane containing 5 cc. of formic acid and stir electromagnetically for thirty minutes. Filter through a Whatman No. 1 filter paper into a 100-cc. volumetric flask. The volume of the solvents will be proportional according to the amounts of active ingredient present. For example, when a sample contains 500 mg. of antibiotic, the amount of formic acid and nitromethane will be doubled. Wash the residue, then make up to volume with the nitromethane. To an aliquot equivalent to 50 mg. of drug add enough nitromethane to total 50 cc., 5 cc. of benzene, 1 cc. of 6% mercuric acetate in glacial acetic acid, and 0.1 cc. of the mixed indicator. Titrate to a green end point with 0.05 *N* perchloric acid in dioxane.

Chlortetracycline (Aureomycin) Hydrochloride Suppositories.—Place as many suppositories as required to yield a solution to contain 200 to 250 mg. of antibiotic in a 150-cc. beaker. Add 50 cc. of benzene and warm on a hot water bath until the suppositories are uniformly dispersed. Stir the mixture electromagnetically and allow to cool. Filter the cooled solution through a medium porosity sintered glass funnel, washing the residue with an additional 50 cc. of benzene. Place the funnel filled with nitromethane and 2 cc. of formic acid in a beaker, then allow the mixture to filter through under the influence of gravity. Repeat with additional nitromethane and the remainder of the formic acid, the total volume having been calculated such that 1 cc. is present for each 50 mg. of drug. Wash the funnel with nitromethane, transfer to a 200-cc. volumetric flask and make up to volume with nitromethane. To an aliquot equivalent to 50 mg. of active constituent add sufficient nitromethane to total 50 cc., 5 cc. of benzene, 1 cc. of 6% mercuric acetate in glacial acetic acid, and 0.1 cc. of the mixed indicator. Titrate to a blue-green end point with 0.05 *N* perchloric acid in dioxane.

DISCUSSION

Tetracycline antibiotics, by virtue of the secondary amine group, can be determined as bases by direct titration with a strong acid such as perchloric. Both the free base and the hydrohalide salt can be assayed, the latter being dependent upon the addition of mercuric acetate solution as reported by Pifer and Wollish (15).

Of the numerous solvents or solvent combinations investigated, the nitromethane-formic acid pair employed by Struli (16), in the titration of basic copolymers of acrylonitrile, was found to be the most satis-

factory, particularly when combined with benzene. The addition of 2% by volume of formic acid to nitromethane appeared to solubilize the compounds completely, and thus eliminated the use of heat which was sometimes found necessary with glacial acetic acid. With some of the antibiotics, addition of formic acid was not required to effect complete solution but in order to maintain uniformity of the solvent system, however, it was used in all instances. With the use of 85–90% formic acid, the maximum amount of water introduced was calculated to be approximately 0.55% by volume, which according to Pifer, *et al.* (15), and Fritz, *et al.* (17), should cause no appreciable decrease in the sharpness of the potentiometric end point. Pifer, *et al.* (18), reported that the addition of a solvent with a low dielectric constant like benzene, chloroform, or dioxane can in many cases contribute to an increase of the potentiometric break at the end point. Of those investigated, benzene proved to be the most satisfactory.

With the proposed solvent mixture, the electrode combination of silver-silver chloride and glass was found to give a better potentiometric break at the end point in comparison to that obtained with calomel and glass. Another factor in favor of the former pair is that it is not necessary to periodically replace the calomel electrode salt bridge which is known to be readily contaminated by the mercuric acetate.

The main difficulty in finding a suitable indicator was caused by the inherent yellow color of the antibiotic, which created a considerable decrease in the sharpness of the color change at the end point. A few of the indicators which appeared to change at the potentiometric end point were: α -naphtholbenzein, quinaldine red, bromothymol blue, and tropacolin 00. However, with all of these, the end-point change was masked by the color of the antibiotic. For example, the usual red of tropacolin 00 at the equivalence point was altered to red-orange, and, to overcome this difficulty, mixed indicators were tried. Methylene blue, which remained unchanged throughout the titration was chosen to provide the background color. Tropacolin 00, when used alone changed from yellow to red-orange, but with the blue background provided by the methylene blue, the corresponding change was from green to purple which appeared to be easier to see visually. However, one could not determine the blank quite as accurately because of the less definite change, namely from blue-purple to red-purple. The mixed indicator which exhibited the most satisfactory result was the combination of methylene blue with quinaldine red where the color transition was from purple to blue, blue-green, or green depending upon the preparation. The determination of the blank was also improved with the change from purple to a definite blue. The proportion of each component in the screened indicator was found to play an important part, the most satisfactory one being outlined under the heading of reagent. Methanol was the solvent of choice, because the color did not fade back as it did to some extent when the indicators were dissolved in glacial acetic acid. The reproducibility of the results show that this solvent system and indicator combination is superior to that of Sideri and Osol (13) which enabled one to carry out only potentiometric determination with some of the compounds.

With chlortetracycline hydrochloride and tetra-

cycline base, the color of the indicator which coincided with the potentiometric end point was blue as shown in Figs. 1 and 2. However, it was noted that an increment of 0.01 cc. of the titrant altered the color from blue to green. For visual determination it was believed that a change from purple to green was more readily detected than the change from purple to blue. Hence, the green end point was selected with the full realization that it was 0.01 cc. of titrant beyond the true end point. It was thought that the error introduced was insignificant and was compensated for by the reproducibility of the results. However, with the tetracycline hydrochloride and oxytetracycline hydrochloride the color of the indicator at the potentiometric end point was green (see Figs. 3 and 4). Therefore, with all

the pure antibiotics, titration was carried beyond the blue to the green color. Potential changes at the equivalence point were found to be approximately 20 to 30 millivolts for each 0.02 cc. of 0.05 *N* perchloric acid. To test the precision of this method 5 to 10 consecutive determinations were carried out and the standard deviations calculated. In addition, a comparison was made between the results obtained by the microbiological and spectrophotometric procedures. From the results recorded in Table I, one can clearly note the close agreements in the recoveries determined by these three methods, especially between the microbiological and the non-aqueous procedures. It is also apparent that the standard deviation is smallest with the nonaqueous method.

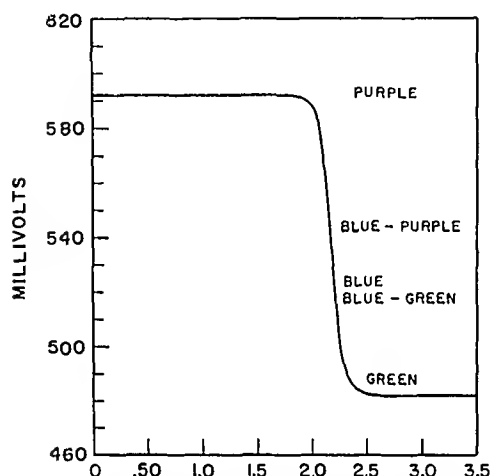


Fig. 1.—Titration of chlortetracycline hydrochloride in nitromethane-formic acid-benzene system.

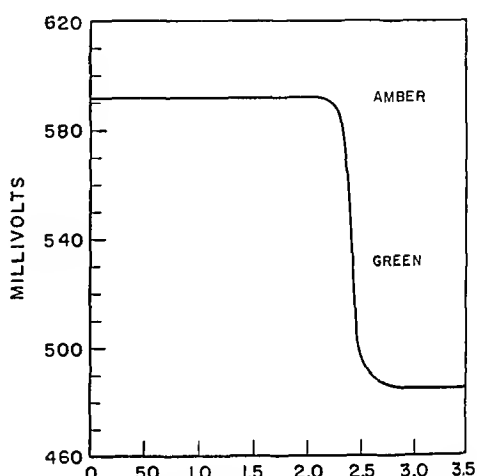


Fig. 3.—Titration of tetracycline hydrochloride in nitromethane-formic acid-benzene system.

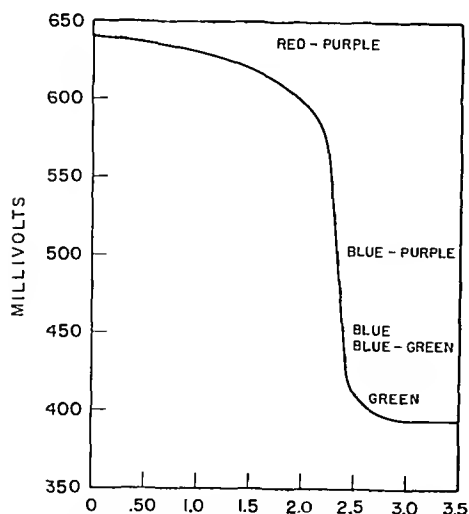


Fig. 2.—Titration of tetracycline base in nitromethane-formic acid-benzene system.

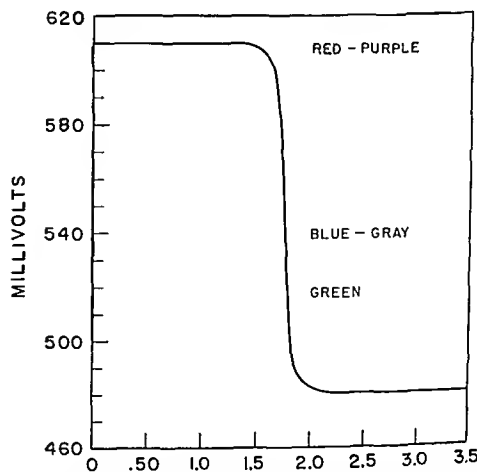


Fig. 4.—Titration of oxytetracycline hydrochloride in nitromethane-formic acid-benzene system.

TABLE I—COMPARISON OF RECOVERIES OF THE PURE TETRACYCLINE ANTIBIOTICS

Compound	Nonaqueous Method	Microbiological Method	Spectrophotometric Method	
Chlortetracycline hydrochloride	99 46 \pm 0.13 (9 det.)	991 μ g./mg. or 99.1% \pm 1.0 (7 det.)		
Tetracycline hydrochloride	99 88 \pm 0.30 (10 det.)	1000 μ g./mg. or 100% (1 det.)	96 3 \pm 1.8 (3 det.)	Acid colorimetric method (1)
Tetracycline base	93 53 \pm 0.46 (9 det.)	1036 μ g./mg. 95.9% \pm 2.3 (6 det.)	92 9 \pm 1.9 (3 det.)	Same as with tetracycline hydrochloride
Oxytetracycline base	91 93 \pm 0.62 Calc. as base $C_{22}H_{24}N_2O_9$ or 99 16 \pm 0.67 Calc. as hydrochloride $C_{22}H_{24}N_2O_9 \cdot HCl$ (6 det.)	929 μ g./mg. or 100.2% \pm 1.6 Calc. as hydrochloride $C_{22}H_{24}N_2O_9 \cdot HCl$ (3 det.)	95 9 \pm 0.0 (3 det.)	Ferric chloride colorimetric method (1)

When the commercial products were investigated, it was found necessary to modify the basic procedure in some instances due to interfering substances like the tablet excipients and ointment bases.

Since magnesium stearate, commonly employed as lubricant in tablets or as diluent in capsules, will react with the perchloric acid, the method required slight modification. This compound was extracted by the nitromethane-formic acid mixture resulting in apparent overestimation of the antibiotic. However, most of these preparations contained the antibiotic as the hydrochloride salt, and therefore, by titrating before the addition of mercuric acetate, the stearate was removed from further competition for the titrant. The volume of titrant consumed after the addition of mercuric acetate is a direct measure of the hydrochloride salt of the antibiotic. Thus, if any of the free base is present along with the hydrochloride salt, the former is not included in the final titration. This may account, in part, for the discrepancies between the microbiological or spectrophotometric methods and the proposed nonaque-

ous procedure (see Table II). To ascertain if the antibiotic was completely extracted during the proposed time of stirring, the procedure was repeated with the residue remaining after filtration. A blank titration showed that the antibiotic had been completely extracted. Unfortunately, the terramycin tablet could not be assayed by this method because the active constituent was present as the free base. It was impossible to determine the volume of the titrant consumed by the stearate because both the stearate and the terramycin base reacted with the perchloric acid without the addition of mercuric acetate. Therefore no results are reported for this preparation.

With the ointments it was found necessary to increase the volume of benzene in order to keep the ointment base in solution. This increase adversely affected the solubility of the antibiotic in the solvent combination and thus it necessitated the doubling of the amount of formic acid. Titration was carried out while hot because the mixture became turbid upon cooling. When the same procedure was re-

TABLE II—COMPARISONS OF THE RECOVERIES OF THE TETRACYCLINE ANTIBIOTICS IN VARIOUS DOSAGE FORMS BY THE NONAQUEOUS, MICROBIOLOGICAL, AND SPECTROPHOTOMETRIC METHODS

Dosage Form	Antibiotic	Nonaqueous Method	Microbiological Method ^a	Spectrophotometric Method ^b
Capsule	Aureomycin hydrochloride	96.01 \pm 0.41 (5 det.)	114 (1 det.)	108.3 \pm 0.2 (3 det.)
	Achromycin hydrochloride	99.72 \pm 0.66 (6 det.)	107 (1 det.)	103.2 \pm 1.1 (3 det.)
	Terramycin hydrochloride	102.7 \pm 0.75 (8 det.) Calc. as free base $C_{22}H_{24}N_2O_9$	103 (1 det.)	105.9 \pm 1.6 (3 det.) Calc. as free base $C_{22}H_{24}N_2O_9$
Tablet	Achromycin hydrochloride	98.96 \pm 0.29 (5 det.)	115 (1 det.)	103.2 \pm 1.1 (3 det.)
Ointment	Aureomycin hydrochloride	115.5 \pm 0.69 (5 det.)	114 (1 det.)	123.2 \pm 0.70 (2 det.)
	Achromycin hydrochloride	118.0 \pm 0.53 (5 det.)	107 (1 det.)	118.6 \pm 1.6 (3 det.)
	Terramycin hydrochloride with polymyxin B sulfate	106.5 \pm 0.24 (5 det.) Calc. as free base $C_{22}H_{24}N_2O_9$	105 (1 det.)	110.5 \pm 1.4 Calc. as free base $C_{22}H_{24}N_2O_9$
Intravenous injection	Terramycin hydrochloride	112.4 \pm 0.38 (5 det.)	93 (1 det.)	117.7 \pm 0.5 (3 det.)
Suppository	Aureomycin hydrochloride	105.8 \pm 0.32 (6 det.)	109.2 (1 det.)	111.5 \pm 2.3 (6 det.)

^a Microbiological assays were carried out by the same techniques as for pure antibiotics following the extraction procedures laid down in the reference.

^b Spectrophotometric assays were carried out by the same methods as for pure antibiotics following the extraction procedures given in the text of Grove and Randall (1). Single exception to this was the intravenous Terramycin hydrochloride, which was analyzed by the ultraviolet method (1).

TABLE III—RECOVERY OF THE PURE ANTIBIOTICS FROM KNOWN CONTROL MIXTURES

Compound	Method	Recovery %
Chlortetracycline hydrochloride	Same as with ointment	99.63
		99.56
		99.97
Tetracycline hydrochloride	Same as with ointment	99.49
		99.51
		100.70
Chlortetracycline hydrochloride	Same as with suppository	99.19
		99.38
		100.0
Control		99.22
Chlortetracycline hydrochloride		99.42
Suppository		99.74

peated with the pure antibiotics, no alteration of recovery or behavior was noted during the titration. Excellent agreement between the basic procedure (Table I) and the control (Table III) appeared to uphold the validity of this method. Potential changes at the equivalence point were found to be approximately 20 to 30 millivolts for each 0.02 cc of titrant. The titration curve for the Achromycin hydrochloride ointment is shown in Fig. 5.

The method was further modified for the terramycin ointment because of the presence of polymyxin B sulfate. The latter compound was believed to cause a slight overestimation. Since the polymyxin B sulfate is insoluble in organic solvents, it was possible to separate the two drugs. The ointment base was first removed by preliminary treatment with benzene, leaving a solid residue comprised of the two antibiotics, and the terramycin hydrochloride was then extracted by nitromethane. Complete extraction of the terramycin hydrochloride was substantiated by repeating the procedure on the white residue remaining in the funnel. To ascertain

that the benzene extract of the base contained no terramycin hydrochloride the procedure was repeated with the base as described in the section on suppositories. Potential changes at the end point were about 20 millivolts for each 0.02 cc of titrant. When the procedure which was employed with the aureomycin and achromycin ointments was applied to this preparation, the recovery tended to be about 0.5% higher than when assayed by the preceding method.

Since the antibiotic was insoluble in benzene, the suppository base was removed by preliminary treatment with this solvent. The antibiotic, which remained as the residue, was then taken up in solution by allowing the solvent mixture to be in contact with the compound. In order to completely extract the antibiotic from the pores of the sintered glass funnel, it was found necessary to prolong the contact of the drug with the solvent and consequently suction was not desirable. Filtration was permitted to proceed by gravity. To ensure that no antibiotic was extracted during the removal of the base, the benzene filtrate was evaporated and the entire procedure was repeated with the residue. Assay of the control suppository prepared according to the manufacturer's formulation and also of the pure compound by the similar method appeared to uphold the validity of the procedure. By comparing Tables I and III one can clearly note the excellent agreement in the recoveries of the pure antibiotic when assayed according to the basic procedure and the modified method for the suppository. The potential changes were approximately 20 to 25 millivolts for each 0.02 cc of titrant. Titration curve for this preparation is shown in Fig. 6.

The Terramycin hydrochloride injection offered no difficulty. Potentiometric changes at the equivalence point were approximately 30 millivolts for each 0.02 cc of titrant as shown in Fig. 7.

The results of these investigations of the commercial preparations are outlined in Table II along with

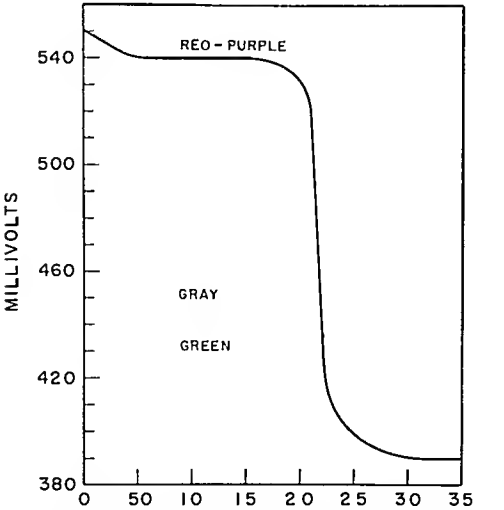


Fig. 5—Titration of Achromycin hydrochloride ointment in nitromethane-formic acid-benzene system

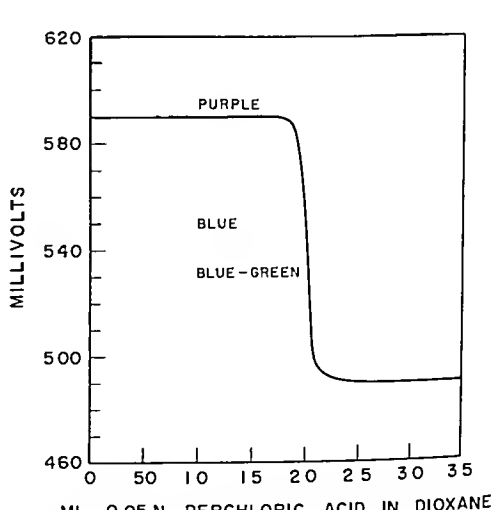


Fig. 6—Titration of Aureomycin hydrochloride suppositories in nitromethane-formic acid-benzene system

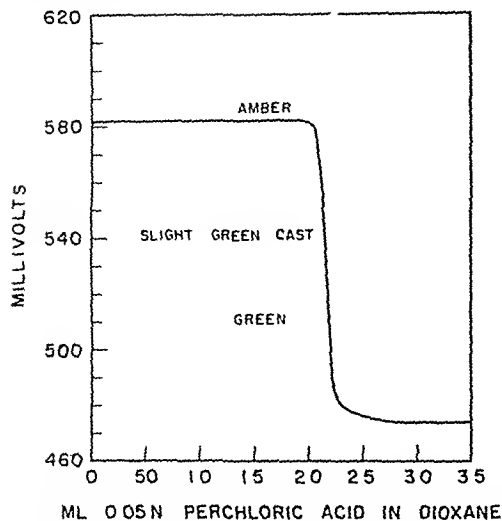


Fig 7—Titration of Terramycin hydrochloride intravenous injection in nitromethane-formic acid-benzene system.

the calculated standard deviations. Generally better agreement appears to occur between replicates with the nonaqueous procedures than with microbiological or spectrophotometric methods. The proposed technique seems to be preferable to that of Sideri and Osol (13) for both the pure compounds and some of their pharmaceutical preparations.

SUMMARY

1. A nonaqueous technique has been devised for the assay of the tetracycline antibiotics which is accurate and can be performed visually.

2. The procedure has been successfully applied to dosage forms including tablets, capsules, ointments, suppositories, and injectables.

REFERENCES

- (1) Grove, D. C., and Randall, W. A. "Assay Methods of Antibiotics" No. 2, 1st ed., Medical Encyclopedia, Inc., New York, 1955, p. 48.
- (2) Monastero, P., Means, J. A., Grenfell, T. C., and Hedger, F. H., *This Journal*, 40, 241 (1951).
- (3) Hiscox, D. J., *ibid.*, 40, 237 (1951).
- (4) Ravin, L. J., and James, A. E., *ibid.*, 44, 215 (1955).
- (5) Levine, J., Garlock, E. A., and Fischbach, H., *ibid.*, 38, 473 (1949).
- (6) Perlman, D., *Science*, 118, 628 (1953).
- (7) Woolford, M. H., and Chuccarelli, F. S., *This Journal*, 45, 400 (1956).
- (8) Chuccarelli, F. S., Giesse, P. V., and Woolford, M. H., *ibid.*, 45, 418 (1956).
- (9) Sakaguchi, T., and Taguchi, K., *Pharm. Bull. (Tokyo)*, 3, 166 (1955); through *Chem. Abstr.*, 50, 2122b (1956).
- (10) Sakaguchi, T., and Hanaki, A., *J. Pharm. Soc. Japan*, 76, 172 (1956); through *Chem. Abstr.*, 50, 7924f (1956).
- (11) Sakaguchi, T., and Hanaki, A., *ibid.*, 76, 176 (1956); through *Chem. Abstr.*, 50, 7924g (1956).
- (12) Kakei, K., Uno, T., and Samejima, M., *ibid.*, 75, 970 (1955).
- (13) Sideri, C. N., and Osol, A., *This Journal*, 42, 683 (1953).
- (14) Ekeblad, P., *J. Pharm. and Pharmacol.*, 4, 636 (1952).
- (15) Pifer, C. W., and Wollish, E. G., *Anal. Chem.*, 24, 300 (1952).
- (16) Streuli, C. A., *ibid.*, 27, 1827 (1955).
- (17) Fritz, J. S., and Fulda, M. O., *ibid.*, 25, 1837 (1953).
- (18) Pifer, C. W., Wollish, E. G., and Schmall, M., *ibid.*, 25, 310 (1953).

The Effects of Some Solid Buffering Agents in Aqueous Suspension on Prednisolone*

By THOMAS CHULSKI and ARLINGTON A. FORIST

The effects of aqueous suspensions of a number of solid buffers on prednisolone at 37.5° have been determined. The steroid was adsorbed by magnesium trisilicate according to the Freundlich relationship. Magnesium oxide suspensions produced a first-order degradation of the steroid side-chain to the etio acid. Aluminum hydroxide, calcium carbonate, and magnesium carbonate were without effect.

RECENTLY, several cortical steroid formulations containing buffering agents have appeared. Granulation procedures frequently used in the manufacture of such preparations involve exposure of the ingredients to moisture and elevated temperatures. In order to predict the effects of such treatment, the behavior of prednisolone ($\Delta^1,4$ -pregnadien-11 β ,17 α ,21-triol-3,20-dione) has been determined in the presence of aqueous suspensions of a number of solid buffers at 37.5°.

EXPERIMENTAL

Materials.—The buffers employed were as follows: (a) magnesium trisilicate Po, U. S. P., (b) magnesium oxide, heavy, U. S. P., (c) aluminum hydroxide, gel dried, U. S. P., (d) calcium carbonate, precipitated, U. S. P., and (e) magnesium carbonate, light, U. S. P. The prednisolone was 99.82% pure by solubility analysis. The blue tetrazolium reagent, 3,3'-dianisole-bis-4,4'-(3,5-diphenyl)-tetrazolium chloride was obtained from the Dajac Laboratories, Leominster, Mass.

Procedure.—In preliminary studies, 500 mg. of the solid buffer were mixed with 15 ml. of an aqueous solution of prednisolone (53–220 μ g./ml.) in a 25-ml. volumetric flask. The flask was attached to a

* Received November 20, 1957, from The Upjohn Company, Kalamazoo, Mich.

The authors are indebted to L. M. Reinecke and associates for the paper chromatographic analyses.

Burrell wrist action shaker mounted over a 37.5° constant temperature bath and the flask and its contents immersed in the bath and shaken for three hours. The solid was permitted to settle for approximately thirty minutes, still in the bath, and the supernatant solution was then withdrawn from the flask through a glass frit by means of vacuum. The resulting filtrate was analyzed for the conjugated carbonyl group of the steroid A ring by ultraviolet spectrophotometry (243 m μ) and for the C₁₇ α -ketol side chain by the modification of the blue tetrazolium procedure of Mader and Buck (1) described below. The solid phase was leached with absolute ethanol and this solution likewise analyzed by the ultraviolet and blue tetrazolium procedures. Suspensions of the buffers without steroid were also carried through the above treatment in order to correct the analyses for any extraneous materials eluted from the solids. The pH of each suspension was determined at 37.5° by means of a Beckman Model G pH meter with glass calomel electrodes.

In the determination of the adsorption isotherm for prednisolone on magnesium trisilicate, equilibration, and filtration were as above. However, only the ultraviolet analysis was applied to the filtrates as preliminary studies had shown adsorption of the steroid but no side chain degradation.

In the kinetics studies of the degradation of the α ketol side chain in the presence of magnesium oxide, reaction mixtures were prepared as above. Shaking was stopped three minutes prior to the removal of each sample. A small portion of the supernatant solution was simultaneously withdrawn and filtered through a glass frit by means of vacuum. An aliquot of the filtrate (0.20–0.50 ml) was immediately removed with a micropipet, the time noted, and the reaction quenched by the addition of the sample to 9.00 ml of absolute ethanol. The resulting solution was analyzed by the modified blue tetrazolium procedure since preliminary studies had indicated degradation of the side chain of prednisolone with no adsorption.

The blue tetrazolium procedure as originally presented by Mader and Buck (1) required ethanolic solutions of the steroid. The modified procedure described below was devised to accommodate the aqueous steroid solutions encountered in these studies. One milliliter of aqueous sample was mixed with 9.00 ml of absolute ethanol followed by 1.00 ml of diluted tetramethylammonium hydroxide (5 ml of 10% aqueous tetramethylammonium hydroxide diluted to 50 ml with absolute ethanol) and 1.00 ml of blue tetrazolium reagent (0.5% in 95% ethanol). The solution was mixed, color development was allowed to proceed for thirty minutes in the dark, and 1.00 ml of glacial acetic acid was added to stabilize the color. Absorbance of the resulting solution was then determined at 530 m μ versus a reagent blank. Under these conditions, color production in the alkaline solution was nearly maximal after thirty minutes, although it continued to develop for two hours. Upon acidification with glacial acetic acid, the color was stable for at least seventy-five minutes as reported by Nowaczynski, Goldner, and Genest (2).

DISCUSSION

The ultraviolet analysis of the filtrates for the un-

saturated carbonyl group of the steroid A-ring indicated the extent of adsorption but did not reveal degradation of the labile side chain. On the other hand, the blue tetrazolium procedure for the α ketol side chain did not differentiate adsorption from degradation. Therefore, to define completely the effects encountered, the systems were analyzed by both techniques.

Typical results for the five buffers studied are presented in Table I. From the ultraviolet analyses it may be seen that adsorption occurred on the magnesium trisilicate. Agreement between the blue tetrazolium and ultraviolet analyses indicates that no degradation was involved. Similar agreement was observed between the blue tetrazolium and ultraviolet analyses of material leached from the solid magnesium trisilicate phase thereby eliminating the possibility of adsorption via degradation. The adsorption isotherm for prednisolone on magnesium trisilicate at 37.5° is shown in Fig. 1. Over the range studied the isotherm was linear and may be expressed according to the Freundlich equation $x/m = 10 c^{1/11}$ where c is the equilibrium concentration (μ g/ml) and x/m is the amount adsorbed (μ g/Gm).

TABLE I—EFFECTS OF SOLID BUFFERING AGENTS ON PREDNISOLONE

Buffer	pH of Suspension	Prednisolone μ g/ml		
		Initial	UV Equilibrium	BT
Magnesium trisilicate	8.9	53	34	33
		220	145	145
Magnesium oxide	10.4	220	223	140
Aluminum hydroxide	7.4	53	55	50
		220	219	225
Calcium carbonate	9.1	53	53	52
		220	220	220
Magnesium carbonate	9.2	220	222	225

Comparison of the ultraviolet and blue tetrazolium analyses in the case of magnesium oxide (Table I) shows that there was no adsorption of steroid but there was extensive side-chain degradation. From Fig. 2 it may be seen that the degradation was first order in steroid and that the pseudo first order constant (k') was independent of the initial steroid concentration and of the steroid-buffer ratio. The high pH of the magnesium oxide suspension (Table I) suggests that the degradation was due to the high alkalinity of the solution. Velluz, Petit, Pesetz, and Berret (3) found that, in the presence of air and alkali, the C₂₀ C₂₁ bond of the α ketol side chain was split with the production of the corresponding etio acid and formic acid and with the consumption of two moles of base and one mole of oxygen. This was confirmed by Herzig and Ehrenstein (4). Paper chromatographic examination of a reaction mixture obtained with magnesium oxide in the present studies gave similar results. Prednisolone (I) was converted predominantly to 3-keto 11 β ,17 α dihydroxy Δ^1 -etiocholenic acid (II) and to a lesser degree to Δ^1 -androsta-11 β -ol-3,17-dione (III). Removal of the steroid side chain to give the corresponding 17 ketosteroid (III) has been reported by Mason (5) and by Wendler and Graber (6) and represents a hydroxide ion catalyzed aldolization occurring under both aerobic and anaerobic conditions.

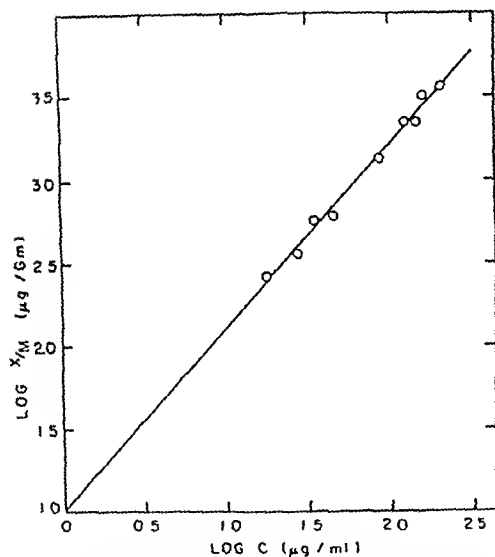
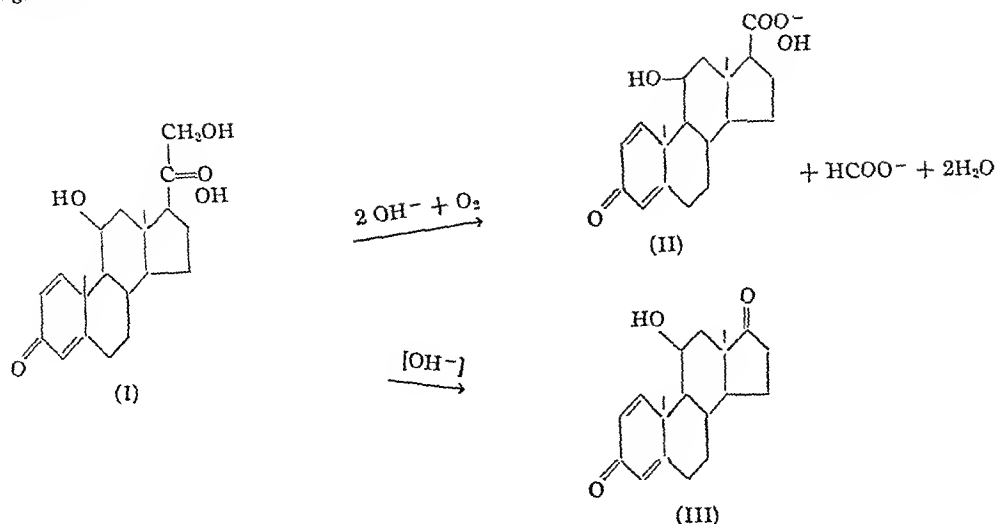


Fig 1—Adsorption isotherm for prednisolone on magnesium trisilicate at 37.5°

Under the conditions employed, aluminum hydroxide, calcium carbonate, and magnesium carbonate were without effect on prednisolone (Table I)

It may be concluded therefore that the cortical steroids similar to prednisolone should not be exposed to materials capable of producing an elevated pH during formulation

SUMMARY

The effects of aqueous suspensions of magnesium trisilicate, magnesium oxide, aluminum hydroxide, calcium carbonate, and magnesium carbonate on prednisolone at 37.5° have been studied. Magnesium trisilicate adsorbed the intact steroid. Magnesium oxide produced an alkaline degrada-

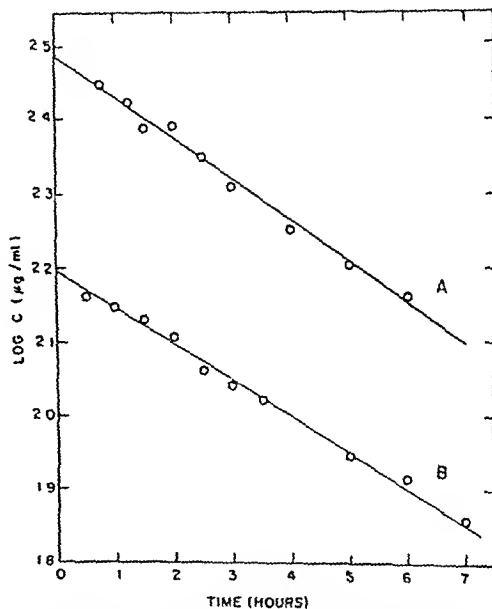


Fig 2—First order plots of the degradation of prednisolone by magnesium oxide suspensions at 37.5° A, initial concentration 310 μg/ml, $k' = 127 \times 10^{-3} \text{ hr}^{-1}$, B, initial concentration 155 μg/ml, $k' = 114 \times 10^{-3} \text{ hr}^{-1}$

tion of the steroid side chain. The other buffers were without effect.

REFERENCES

- (1) Mader W J and Buck, W W, *Anal Chem*, 24, 666(1952)
- (2) Nowaczynski, W, Goldner M, and Genest J, *J Lab Clin Med* 45, 818(1955)
- (3) Velluz L, Petit A, Pesez M and Berret R, *Bull soc chim France* 123(1947)
- (4) Herzog P T and Ehrenstein M, *J Org Chem*, 16, 1050(1951)
- (5) Mason H I, *Proc Mayo Clin* 13, 235(1938)
- (6) Wendler N I and Graber R P, *Chemistry and Industry* 519(1956)

Copper Complexes of Aromatic Dithiocarbamates and Their Antifungal Activity*

By WILLIAM O. FOYE, IVAN B. VAN de WORKEEN, Jr.,† and JOSEPH D. MATTHES

Phenyl and 2-pyridyl dithiocarbamate salts and esters have been prepared and found to form stable complexes, probably chelates, with copper (II) ion. The aromatic dithiocarbamates showed a low order of antifungal and antibacterial activity, but their copper complexes were practically inactive. This finding does not support previous postulations that antifungal dithiocarbamates exert their fungitoxicity as toxic copper chelates.

INVESTIGATIONS at the duPont Company in 1931 showed that derivatives of dithiocarbamic acid possessed antifungal and antibacterial properties, and a patent reporting the antifungal activity of thiuram disulfides was issued to Tisdale and Williams (1) in 1934. The activity of several dithiocarbamic acid derivatives against fungi pathogenic to man was noted by Hall in 1938 in an unpublished report, and was later studied by Miller and Elson (2). Within twenty years of their discovery, the dithiocarbamates had become widely used for controlling plant pathogens (3).

The suggestion that metal chelation is a mechanism of biotoxic action for antifungal agents was made by Zentmyer (4) in 1943. He observed that the fungicidal action of oxine (8-hydroxyquinoline) was reversed by zinc ion, and at low pH, where the chelating ability of oxine was weak, the oxine showed weak fungicidal activity. Shortly after, Albert (5) demonstrated conclusively that the bactericidal action of oxine is due to the formation in the cell of a toxic 1:1 chelate with iron or copper. The copper chelate of oxine has subsequently found commercial use mildew-proofing textiles and paint. Goksøyr (6) has also postulated that the fungicidal action of sodium dimethyldithiocarbamate is due to the formation of toxic 1:1 chelates with copper and zinc.

To test further the hypothesis that formation of toxic metal chelates is a mechanism of fungicidal action, a series of dithiocarbamate salts and esters has been prepared in order to compare the activities of chelated and nonchelated derivatives. Aromatic dithiocarbamates were selected for this purpose and also to determine the relative

fungicidal properties of aromatic dithiocarbamates, which have not been looked at to any extent for fungicidal effects.

METHODS OF PREPARATION

The ammonium salt of phenyldithiocarbamic acid was easily prepared by the method of Dains, Brewster, and Olander (7), and esters were obtained by standard procedures. Use of this method to obtain 2-pyridyldithiocarbamates was unsuccessful, however, and a review of the literature showed that heterocyclic dithiocarbamates have not received much attention. In a review of the chemistry of dithiocarbamates by Chabrier and Nachmias (8), for instance, no heterocyclic derivatives were mentioned. Only two references found in the literature described the preparation of pyridyl derivatives, and in only one of these was the product isolated, but not characterized. This product was a mixture believed to contain the 4-aminopyridyl salt of 4-pyridyldithiocarbamic acid (9), in analogy to the general formation of aliphatic dithiocarbamates.

Among the methods attempted for the preparation of 2-pyridyldithiocarbamic acid derivatives was that of Camps (9) for the suspected salt of the 4-isomer, using 2-aminopyridine, carbon disulfide, and ethanol, and also that of Klarer and Behnisch (10), who mentioned the intermediate formation of the potassium salt of 2-pyridyldithiocarbamic acid. Both of these methods failed, as did two procedures in which aniline has been used to obtain phenyldithiocarbamic acid. The latter two required the use of alcoholic potassium hydroxide or aqueous ammonia in the presence of carbon disulfide and the aromatic amine.

The desired reaction was accomplished by first forming the sodium salt of 2-aminopyridine in non-aqueous media with sodamide, according to Chichibabin (11), and the isolated sodium salt was then suspended in benzene and allowed to react with carbon disulfide. The product was found by analysis to be the 2-aminopyridyl salt of 2-pyridyldithiocarbamic acid, which is analogous to the product described by Camps (9) from 4-aminopyridine. Esters of this acid, which were obtained by reaction of the salt with organic halides, were difficult to purify but did give nitrogen analyses approximating theoretical. The dithiocarbamate salts and esters prepared and their properties are listed in Table I.

The reaction of copper ion with the salts and esters described above was carried out to determine the

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TABLE I.—AROMATIC DITHIOCARBAMATES

		$\text{Ar}-\text{NH}-\overset{\text{S}}{\underset{\text{ }}{\text{C}}}-\text{S}-\text{R}$				Analysis, % ^b	
Ar	R	M. P. ^a	Yield, %	Formula	Calcd.	Found	
2-Pyridyl	2-Pyridylammonium	...	86	C ₁₁ H ₁₂ N ₄ S ₂	C: 49.98	50.41	
2-Pyridyl	Ethyl	Oil	50	C ₈ H ₁₀ N ₂ S ₂	H: 4.60	5.27	
2-Pyridyl	<i>p</i> -Nitrophenyl	128–130°	10	C ₁₂ H ₉ N ₃ O ₂ S ₂	N: 14.14	13.87	
Phenyl	Ammonium	92–93°	73	C ₇ H ₁₀ N ₂ S ₂	N: 14.18	13.67	
Phenyl	Ethyl	58–60°	41	C ₉ H ₁₁ NS ₂	N: 15.06	14.80	
Phenyl	<i>p</i> -Nitrophenyl	88–90°	50	C ₁₂ H ₁₀ N ₂ O ₂ S ₂	N: 7.11	7.21	
					N: 9.62	9.48	

^a The melting points were taken on a Fisher-Johns block and are uncorrected.

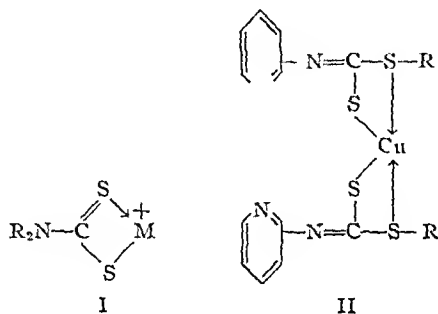
^b The carbon-hydrogen analysis was obtained from the Weiler and Strauss Microanalytical Laboratory, Oxford, England. The nitrogen analyses were done by the Kjeldahl procedure, using selenized Hengar granules as catalyst.

possibility of complex or chelate formation. This was done in a suitable solvent with aqueous copper sulfate, and in each case complexing was indicated by a marked drop in pH, and formation of a colored precipitate in the case of the salts. Copper complexes soluble in the organic media used were obtained from the esters. Analysis indicated that complexes having a 2:1 ratio of dithiocarbamic acid to copper were obtained from the salts and the *p*-nitrophenyl esters, and complexes with a 1:1 ratio were obtained from the ethyl esters. The physical properties of the complexes prepared are listed in Table II.

The structures of 1:1 metal chelates of dithiocarbamic acids have been determined by Chatt, *et al.* (12), and pictured as four-membered rings (structure I) with several resonating forms possible. The 2:1 copper complexes can accordingly be represented by structure II, although open-chain complexes with the copper coordinated to two sulfurs or possibly two nitrogens are also possible and would also cause a drop in pH on formation. Complexing by means of the pyridine nitrogen is not postulated for these compounds, since the phenyl derivatives gave the same results as the pyridyl.

BIOLOGICAL RESULTS

Antifungal and antibacterial tests were carried out at the Lilly Research Laboratories, and the results of positive activity are shown in Table III. It is apparent that the salts of phenyl- and 2-pyridyldithiocarbamic acids have essentially the same activity against microorganisms; while the



degree of activity differed slightly in several cases, these compounds generally showed activity toward the same organisms, and both were also negative toward a number of other organisms.

The copper chelates of these acids, on the other hand, showed essentially no activity toward the test organisms. Only *U. avenae* was inhibited by the chelates at a concentration of 200 mcg./ml. but not at 50 mcg./ml. (The esters and their copper derivatives have not as yet been tested.) This result implies that dithiocarbamates do not exert their fungicidal action in the form of toxic copper chelates, although chelation of essential copper in the organisms is still a plausible mechanism of action. Further such comparisons should be made with other dithiocarbamates, however, although the comparative results might be obscured somewhat with weaker chelators or stronger antifungal agents.

TABLE II.—COPPER COMPLEXES OF THE DITHIOCARBAMATES

		$(\text{Ar}-\text{N}=\overset{\text{S}}{\underset{\text{ }}{\text{C}}}-\text{SR})_2\text{Cu}$							
Ar	R	pH Drop ^a	Yield, %	M. P. ^b	Color	Formula	N Analysis, %	Calcd.	Found
2-Pyridyl	H	2 6.6	74	115°(dec.)	Green-black	C ₁₂ H ₁₀ N ₄ S ₄ Cu	13.93	13.42	
2-Pyridyl	C ₂ H ₅	1 3.4	40	88–90°	Green-black	C ₈ H ₁₀ N ₂ S ₂ Cu	9.40	9.94	
2-Pyridyl	C ₆ H ₄ NO ₂	2 2.0	48	132–134°	Yellow-green	C ₂₁ H ₁₆ N ₄ O ₄ S ₄ Cu	12.96	11.76	
Phenyl	H	2 3.0	84	110°(dec.)	Yellow	C ₈ H ₁₂ N ₂ S ₂ Cu	7.00	6.96	
Phenyl	C ₂ H ₅	1 3.0	56	50–52°	Yellow-green	C ₉ H ₁₁ NS ₂ Cu.2H ₂ O	4.71	5.10	
Phenyl	C ₆ H ₄ NO ₂	2 2.5	50	103–105°	Red	C ₂₆ H ₁₈ N ₄ O ₄ S ₄ Cu	8.67	8.53	

^a The drop in pH was recorded during the addition of copper sulfate.

^b The melting points were taken on a Fisher-Johns block and are uncorrected.

^c The nitrogen analyses were done by the Kjeldahl procedure, using selenized Hengar granules as catalyst.

TABLE III.—ANTIMICROBIAL TESTING OF THE DITHIOCARBAMATE SALTS^a

Test Organism	Inhibitory Concn, mcg/ml ^b	
	Phenyl Deriv. ^c	2-Pyridyl Deriv. ^d
<i>Staphylococcus albus</i>	200	200
<i>Bacillus subtilis</i>	50	50
<i>Mycobacterium tuberculosis</i>	50	50
<i>Klebsiella pneumoniae</i>	100	
<i>Trichophyton interdigitale</i>	100	100
<i>Shigella paradysenteriae</i>		100
<i>Xanthomonas phaseoli</i>	25	25
<i>Monilinia fructicola</i>	100	50
<i>Ustilago avenae</i>	12 5	12 5
<i>Alternaria solani</i>	<6 25	<6 25
<i>Fusarium moniliforme</i>	200	..
<i>Colletotrichum gossypii</i>	50	100
<i>Ascochyta imperfecta</i>	25	200
<i>Sclerotinia bataticola</i>	50	50

^a Carried out at the Lilly Research Laboratories by D. Fleming

^b The agar dilution technique was used, the bacteria being observed for forty-eight hours and the bacterial and fungal plant pathogens being observed for seventy-two hours. Those organisms having no inhibitory levels indicated were not affected by 200 mcg/ml

^c Ammonium phenyldithiocarbamate

^d 2-Pyridylammonium 2-pyridyldithiocarbamate

EXPERIMENTAL

Ammonium Phenyldithiocarbamate.—The procedure of Dains, Brewster, and Olander (7) was used. A 73% yield of white powder was obtained which melted at 92–93°. Losanitsch (13) first described this compound as decomposing at 100°. Dains, *et al*, list no physical constants.

Ethyl Phenyldithiocarbamate.—Three grams (0.016 mole) of ammonium phenyldithiocarbamate and 75 ml (1 mole) of ethyl bromide were stirred at room temperature for four hours. The mixture was filtered, and the filtrate was distilled under reduced pressure. The remaining semisolid was taken up in ether and allowed to crystallize, giving 1.25 Gm (41%) of yellow-green crystals; m p 58–60°.

p-Nitrophenyl Phenyldithiocarbamate.—One gram (0.005 mole) of ammonium phenyldithiocarbamate was dissolved with gentle heating in 30 ml of distilled water. Ethyl alcohol (20 ml) and p-nitrochlorobenzene (1.0 Gm, 0.006 mole) were added, and the solution was refluxed for one hour. A light yellow powder precipitated after the solution cooled, giving 0.8 Gm (50%) of product melting at 88–90°.

2-Pyridylammonium 2-Pyridyldithiocarbamate.—A solution of 12 Gm (0.31 mole) of sodamide in 50 ml of benzene was treated dropwise with 25 Gm (0.27 mole) of 2-aminopyridine dissolved in 175 ml of benzene with warming. The mixture was refluxed for two hours and allowed to cool. A solution of 17 ml (0.28 mole) of carbon disulfide in 50 ml of benzene was then added dropwise with stirring, and the resulting solution was refluxed with stirring (mercury seal) for four hours and allowed to stand overnight. The yellow precipitate which appeared

was washed with ether and dried. The yield was 30 Gm. (86%). Erratic results were obtained from Kjeldahl determinations on this product using either selenium or mercuric oxide as catalyst, but a carbon-hydrogen combustion analysis gave values approaching theoretical. The product was contaminated with sodium ion, however.

Ethyl 2-Pyridyldithiocarbamate.—Three grams (0.011 mole) of the 2-pyridyldithiocarbamate salt and 75 ml (1 mole) of ethyl bromide were stirred at room temperature for four hours. The mixture was filtered, and the filtrate was distilled under reduced pressure. The red, oily liquid remaining was dissolved in ether, which was partially evaporated. A yield of 1.5 Gm. (68%) of red oil was obtained which decomposed on attempted distillation under reduced pressure.

p-Nitrophenyl 2-Pyridyldithiocarbamate.—One gram (0.005 mole) of the 2-pyridyldithiocarbamate salt was dissolved with gentle heating in 30 ml of distilled water. Ethyl alcohol (20 ml) and p-nitrochlorobenzene (10 Gm., 0.006 mole) were added, and the solution was refluxed for one hour. A green powder precipitated after the solution cooled, giving 0.15 Gm. (10%) of product melting at 128–130°.

Chelation with Copper (II) Ion.—The following procedure is representative. To a filtered solution of 10 Gm (0.05 mole) of ammonium phenyldithiocarbamate in 75 ml of water was added gradually, with stirring, a solution of 4 Gm. (0.025 mole) of copper sulfate in 50 ml of water. During the reaction the pH dropped from 8.3 to 5.3. A yellow precipitate was collected, washed with water, and allowed to dry at room temperature. The yield was 9.0 Gm. (84%); and the product decomposed at 110°.

In the corresponding reaction with the esters, an organic solvent was used—acetone for the ethyl esters and ethanol for the p-nitrophenyl esters. After treatment with aqueous copper sulfate solution in which a minimum amount of water was used, the unreacted copper sulfate was removed by filtration. The filtrate was then allowed to evaporate to dryness to yield the complexes.

REFERENCES

- (1) Tisdale, W. H., and Williams, I., U. S. pat. 1,972,961 (1934).
- (2) Müller, O. R., and Elson, W. O., *J. Bacteriol.*, **57**, 47 (1949).
- (3) Horsfall, J. G., "Principles of Fungicidal Action," Chronica Botanica Co., Waltham, Mass., 1956, p. 171.
- (4) Zentmyer, G. A., *Phytopathology*, **33**, 112 (1913), *Science*, **100**, 291 (1941).
- (5) Albert, A., Gibson, M. I., and Rubbo, S. D., *Brit. J. Exptl. Pathol.*, **34**, 119 (1953).
- (6) Gokspyr, J., *Nature*, **175**, 820 (1955).
- (7) Dains, F. B., Brewster, R. O., and Olander, C. P., "Organic Syntheses," Coll. Vol. I, 2nd ed., John Wiley and Sons, Inc., New York, 1941, p. 447.
- (8) Chabrier, P., and Nachmias, G., *Bull. soc. chim. France*, **17D**, 51 (1950).
- (9) Camps, R., *Arch. Pharm.*, **240**, 315 (1902).
- (10) Klarer, J., and Belinisch, R., German pat. 832,891 (1952).
- (11) Chichibabin, A. E., Konovalova, R. A., and Konovalova, A. A., *Ber.*, **54**, 811 (1921).
- (12) Chatt, J., Duncanson, L. A., and Venanzi, I. M., *Nature*, **177**, 1042 (1956).
- (13) Losanitsch, S. M., *Ber.*, **24**, 3021 (1891).

Chemical Investigation of *Daemia extensa* R. Br.[†]

By S. PATTABI RAMAN and A. K. BARUA

A chemical study of *Daemia extensa* is described. From the petroleum ether extract of the whole plant, three triterpene compounds and a sterol have been isolated and characterized.

DAEMLA EXTENSA R Br (syn *Pergularia extensa* N E Br) belongs to the family *Asclepiadaceae* and it grows wild in India. It is extensively used in the indigenous system of medicine in India (1). From the above plant, Gupta, *et al* (2), described a pharmacologically active glucosidic bitter principle having action on uterus comparable with that of pituitrin. Dutta and Ghosh (3) reported the isolation of four sterols having m p's 172–76°, 163°, 157°, and 70–80°, respectively, from the same plant. The bitter principles could not, however, be obtained in a pure state, nor were the sterols characterized by them. In view of the important physiological action of the drug, a systematic chemical investigation of the whole plant has been undertaken by us.

The petroleum ether extract of the plant on chromatographic resolution over Brockmann's alumina gave three triterpene compounds, α -amyrin acetate, lupeol acetate, and lupeol and also a sterol which could be identified as β -sitosterol. It has not yet been possible to crystallize the bitter fractions obtained from the benzene, chloroform, and alcoholic extracts of the plant and attempts are being made for the same.

EXPERIMENTAL

Air dried powdered drug (whole plant, 700 Gm) was Soxhleted with petroleum ether (b p 60–80°). The volume of the extract was reduced to about 100 cc and was adsorbed on a column of Brockmann's alumina (45 cm \times 2 cm). The column was then eluted successively with petroleum ether (b p 60–80°, 1,000 cc), petroleum ether benzene mixture (1:1, 600 cc), benzene (400 cc), benzene chloroform mixture (1:1, 200 cc), and finally with methanol. The petroleum ether eluate, on removal of the solvent and crystallization of the residue from chloroform-methanol mixture, gave almost white amorphous solids (5.2 Gm, Fraction A). The petroleum ether-benzene and benzene eluates, on removal of

the solvent, left residues which on crystallization from aqueous ethanol gave colorless crystalline compounds of the same m p, 152–164° (0.8 Gm total, Fraction B). The benzene-chloroform and chloroform eluates gave residues which on crystallization from aqueous ethanol gave almost colorless flakes of the same m p, 131–135° (1 Gm total, Fraction C). The methanol eluate left negligible amount of residue.

Fraction A was taken up in benzene (15 cc) and was adsorbed on a column of Brockmann's alumina (16 cm \times 3 cm) and was eluted with petroleum ether (b p 40–60°, 800 cc), collecting 25 cc portions. The first ten fractions yielded a white amorphous mass (3 Gm, m p 115–137°, Fraction D) whereas the subsequent fractions had the extended m p 140–165° (1.2 Gm, Fraction E). The latter fraction on repeated crystallization from chloroform-methanol mixture gave colorless needles, m p 213–215°, $[\alpha]_D^{25} + 47.8^\circ$ (in chloroform) which did not depress the melting point of an authentic sample of lupeol acetate.

Anal—Calcd for $C_{50}H_{80}O_2$: C, 81.99%, H, 11.12%. Found: C, 81.96%, H, 11.3%.

The above product on hydrolysis with ethanolic caustic potash (10%) gave colorless needles from ethanol, m p 211–212°, which depressed the m p of the parent compound but not that of lupeol.

Anal—Calcd for $C_{50}H_{80}O$: C, 84.44%, H, 11.81%. Found: C, 84.32%, H, 11.9%.

It gave a benzoate, m p 263–264°, which was found to be identical with lupeol benzoate by mixed m p determination.

Anal—Calcd for $C_{57}H_{84}O_2$: C, 83.7%, H, 10.3%. Found: C, 83.69%, H, 10.22%.

Fraction D on rechromatography and crystallization from chloroform-methanol gave shining leaflets, m p 222–223°, $[\alpha]_D^{25} + 79^\circ$ (in chloroform), which showed no change in melting point when admixed with an authentic sample of α -amyrin acetate.

Anal—Calcd for $C_{57}H_{84}O_2$: C, 81.99%, H, 11.12%. Found: C, 81.95%, H, 11.25%.

On hydrolysis with ethanolic caustic potash (10%) it gave a compound m p 184–185°, crystallizing as needles from ethanol, which was shown to be identical with α -amyrin by mixed m p determination.

Anal—Calcd for $C_{50}H_{80}O$: C, 84.44%, H, 11.81%. Found: C, 84.3%, H, 11.9%.

This gave a benzoate m p 194–196°, identical with α -amyrin benzoate.

Anal—Calcd for $C_{57}H_{84}O_2$: C, 83.7%, H, 10.3%. Found: C, 83.81%, H, 10.38%.

Fraction B was directly benzoylated and chromatographed over active alumina when a fraction, m p 261–262°, was obtained in major yield. This was shown to be lupeol benzoate by mixed m p determination.

Anal—Calcd for $C_{52}H_{84}O_2$: C, 83.7%, H, 10.3%. Found: C, 83.69%, H, 10.22%.

This on hydrolysis gave lupeol. Thus fraction B is shown to contain lupeol in the free state.

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Fraction C on repeated crystallization from aqueous ethanol gave shining leaflets, m. p. 136–137°, $[\alpha]_D^{25} - 35.5^\circ$ (in chloroform). Admixture with an authentic sample of β -sitosterol showed no change in m. p.

Anal.—Calcd. for $C_{29}H_{50}O$: C, 83.99%; H, 12.15%. Found: C, 83.71%; H, 12.0%.

With pyridine and acetic anhydride at 100°, it gave an acetate m. p. 127–128°, $[\alpha]_D^{25} - 41.5^\circ$ (in chloroform).

Anal.—Calcd. for $C_{31}H_{52}O_2$: C, 81.52%; H, 11.44%. Found: C, 81.6%; H, 11.51%.

The sterol also furnished a benzoate by the usual method, m. p. 143–144°, which did not depress the m. p. of an authentic sample of β -sitosterol benzoate.

Anal.—Calcd. for $C_{36}H_{54}O_2$: C, 83.34%; H, 10.49%. Found: C, 83.32%; H, 10.35%.

SUMMARY AND CONCLUSIONS

The petroleum ether extract of *Daemia extensa* R. Br., has been found to contain α -amyrin acetate, lupeol acetate, lupeol, and β -sitosterol. It appears that the sterols obtained by the previous workers were not homogeneous compounds but mixtures of the above substances.

REFERENCES

- (1) Kirtikar, K. R., and Basu, B. D., "Indian Medicinal Plants," Vol. 2, Labit Moban Basu, Allahabad, India, 1933, p. 1616.
- (2) Gupta, J. C., Roy, P. K., and Dutta, A. T., *Indian J. Med. Res.*, 34, 181(1946).
- (3) Dutta, A., and Ghosh, S., *THIS JOURNAL*, 36, 250 (1947).

Bromide Intoxication II. Biochemical and Hematological Aspects*

By IRA ROSENBLUM and THOMAS L. HAWKINS, Jr.

Progressive bromide intoxication in dogs was shown to be accompanied by a decrease in the volume of the extracellular space and in the terminal stages by hemoconcentration. Mild and transient anemia and leukocytosis occurred in a number of dogs and the sedimentation rate was accelerated in almost all the animals.

DESPITE the fact that chronic bromide intoxication is regarded as being largely neurointoxication, there is little evidence which excludes the possibility that other systems are affected. In a comprehensive review of the clinical literature of bromide intoxication through 1940 (1) there was no mention of the state of liver and kidney function, electrolyte levels, acid-base equilibrium, etc. Since then, Clark, *et al.* (2), have examined the effects of small doses of bromide on some of these systems in animals. Experiments carried out in this laboratory (3) have demonstrated that dogs intoxicated chronically with varying doses of bromide become emaciated, lose weight, and otherwise show signs of systemic dysfunction although signs of neurointoxication are absent. This would support the possibility that bromide disturbs the function of systems other than the central nervous

system. The present experiments were performed to assess the function of some of these systems during chronic bromide intoxication.

METHODS

Adult mongrel dogs of both sexes were used. These animals were treated with a standard veterinary vermifuge and inoculated against canine distemper and hepatitis prior to the start of the experiments. They were kept in individual metabolism cages during the entire experimental period and maintained on a diet of commercial dog food, 40 Gm./Kg. of body weight per day, with water available at all times.

Because each dog was to serve as its own control in these experiments, biochemical and hematological measurements were made at least twice on each dog before administration of bromide was begun. The course of experimental bromide intoxication in dogs produces signs which resemble those seen in man. These have been reported in detail elsewhere (3). In these experiments two procedures were used to produce bromide intoxication. In one group of 4 dogs, intoxication was produced rapidly by administering an initial dose of 400 mg. of sodium bromide per Kg. of body weight per day. This dose was increased once after three weeks to 500 mg. of sodium bromide per Kg. of body weight per day. These dogs were used in one hematological experiment. Death occurred rapidly in this group and all were dead by the end of the sixth week of administration of sodium bromide. Slow intoxication was produced in three groups, each of four dogs, by giving 200 mg. of sodium bromide per Kg. of body weight per day initially. This dose was in-

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creased every six weeks by 100 mg. of sodium bromide per Kg. of body weight per day until one of the animals died. The maximum dose of sodium bromide which was reached by this procedure was 600 mg. per Kg. of body weight per day. These groups were used in both the biochemical and hematological experiments.

Biochemical Methods.—Blood glucose was determined by the method of Nelson and Somogyi using zinc sulfate-barium hydroxide to deproteinize the blood. Urea was converted to ammonia with urease and the nitrogen determined spectrophotometrically using Nessler's reagent. The CO_2 combining power of serum was measured manometrically by the method of Peters and Van Slyke.

Bromsulfalein retention was measured forty-five minutes after injection of the dye. Serum Na and K were measured using a Beckman flame photometer with an internal lithium standard. References to these foregoing procedures may be found in Hawk, Oser, and Summerson (4). Serum inorganic phosphorus was measured by the method of Gomori (5), plasma volume was measured by the method of Gibson and Evans (6), and the thiocyanate space was measured by the method of Gregerson and Stewart (7) using Bowler's method for determining thiocyanate (8). Serum calcium was determined by the method of Kibrich, *et al.* (9), and blood bromide was determined by the method of Brodie and Friedman (10). Urinary coproporphyrins were measured by the fluorometric method of Schwartz, *et al.* (11).

Hematological Methods.—Measurements were made on blood drawn from the jugular vein using heparin as an anticoagulant. Single erythrocyte and leukocyte counts were made using one blood pipet and the Neubauer hemocytometer. Differential leukocyte counts were made on slides stained by Wright solution. Reticulocyte counts were made by the "dry" method using brilliant cresyl blue as the stain. Hemoglobin determinations were made with the Fisher Electro-Hemometer. Hematocrit and "corrected" sedimentation rate were measured by Wintrobe's method (12). Bone marrow aspirations were made with an 18-gauge needle from a spinous process and were processed according to Propp's method (13).

RESULTS

Biochemical.—The biochemical values of the control period are compared to those at seven and twenty weeks after administration of sodium bromide in Table I. Thereafter measurements were obtained from only one dog in each group and so could not serve as a basis for comparison with the control values.

An important sign of bromide intoxication in dogs is the appearance of emaciation and weight loss on undiminished food intake. The effect occurs in most dogs at doses of bromide which cause no other signs of toxicity. One cause for this change appears to be a reduction in the volume of the extracellular space. In this group of dogs, the mean control value for the extracellular (thiocyanate) space was 322 ± 39 ml./Kg.; after the seventh week of ingestion of bromide it had fallen to 225 ± 27 ml./Kg. The plasma volume did not however change significantly during this time and the serum electrolytes

TABLE I.—AVERAGE BIOCHEMICAL VALUES IN DOGS BEFORE AND AFTER ADMINISTRATION OF SODIUM BROMIDE (SLOW INTOXICATION)

	Initial 4	7 Wk. ^{b,c} 3
No. of Dogs		
Blood Br, meq./L.	0.7 ± 0.1^a	52.8 ± 5.6^a
Serum Na, meq./L.	147 ± 1	142 ± 1
Serum K, meq./L.	4.1 ± 0.1	4.4 ± 0.1
Plasma vol., ml./Kg.	42.2 ± 3.8	45.0 ± 4.7
SCN space, ml./Kg.	322 ± 39	225 ± 27

^a Standard deviation of mean.

^b Only one dog survived after the seventh week.

^c The dose of NaBr at this time was 300 mg./Kg. of body weight per day.

were likewise unaltered. Changes in plasma volume and serum sodium and potassium do sometimes occur during bromide intoxication, especially during the terminal stages when the animals are no longer able to eat or drink. At that time, serum sodium and potassium are elevated, probably due to hemoconcentration since the plasma volume is reduced and the hematocrit values increased.

Emaciation may also be the result of derangements in metabolism. The liver and kidney are important organs which are concerned with the metabolism of protein and carbohydrate so that dysfunction of these organs might well lead to emaciation and weight loss. Several functions of these organs were studied in a group of four dogs. These studies indicate that neither bromsulfalein retention nor blood urea nitrogen were changed significantly during bromide intoxications. For example, the mean control value for bromsulfalein retention was $4 \pm 3\%$ and after twenty weeks of ingestion of sodium bromide it was $3 \pm 2\%$. The mean control blood urea nitrogen was 16.7 ± 2.1 mg./100 ml. and after twenty weeks of ingestion of sodium bromide it was 14.7 ± 2.9 mg./100 ml.

It is also evident that these organs were able to contribute to the maintenance of the blood glucose at about the control level (i. e., control blood glucose, 84 ± 10 mg./100 ml., after twenty weeks, 80 ± 10 mg./100 ml.). Other values which were essentially unchanged after twenty weeks of ingestion of sodium bromide were serum calcium, serum phosphorus, serum carbon dioxide combining power, and total urinary coproporphyrins.

Hematological.—The hematological values of the control period are compared to those at seven and nineteen weeks after administration of sodium bromide in Table II, in dogs which underwent slow intoxication. There was little difference between the responses of those dogs subjected to rapid intoxication and those slowly intoxicated and they will be considered together.

There is little definite information in the literature about the status of the hematopoietic system during bromide intoxication. When high blood bromide levels are encountered in cases of human bromide intoxication, the changes observed in the hematopoietic system are equivocal (14). However it has been suggested that both mild anemia and

TABLE II—AVERAGE HEMATOLOGICAL VALUES IN DOGS BEFORE AND AFTER ADMINISTRATION OF SODIUM BROMIDE (SLOW INTOXICATION)

	Group 1		Group 2	
	Initial	7 Wk b,d	Initial	19 Wk c,e
No of dogs	4	3	4	3
Blood Br, meq /L	0 8 ± 0 1 ^a	52 8 ± 5 6 ^a	0 7 ± 0 1 ^a	52 3 ± 9 2 ^a
Erythrocytes × 10 ⁶ /cu mm	5 8 ± 0 5	5 0 ± 1 3	6 8 ± 0 7	6 1 ± 0 1
Reticulocytes, %	0 4 ± 0 3	0 8 ± 0 5		
Hemoglobin, Gm /100 ml	11 8 ± 0 6	11 1 ± 1 9	14 8 ± 1 1	13 1 ± 0 6
Hematocrit, %	41 ± 3	32 ± 6	47 ± 5	39 ± 1
Leukocytes × 10 ³ /cu mm	15 6 ± 3 6	25 1 ± 17 0	12 5 ± 1 9	14 2 ± 3 1
Band Neutrophils, %	8 ± 2	11 ± 7	8 ± 4	5 ± 1
Mat Neutrophils, %	68 ± 4	71 ± 1	65 ± 3	71 ± 6
Lymphocytes, %	16 ± 5	12 ± 5	15 ± 5	14 ± 6
Monocytes, %	2 ± 1	5 ± 2	8 ± 5	5 ± 1
Eosinophils, %	5 ± 4	5 ± 2	5 ± 4	6 ± 1
Sedimentation Rate, mm /hour	4 ± 1	31 ± 11	8 ± 5	23 ± 1

^a Standard deviation of mean^b Only one dog survived after the seventh week^c Only two dogs survived after the nineteenth week^d The dose of NaBr at this time was 300 mg /Kg of body weight per day^e The dose of NaBr at this time was 500 mg /Kg of body weight per day

transitory leucocytosis does occur (14), and our observations would seem to bear this out

Erythrocytes.—Some decrease in erythrocytes was noted in dogs in both groups during the course of intoxication. These animals showed concomitant decreases in hemoglobin and hematocrit values. The greatest loss of hemoglobin was approximately 6 Gm /100 ml which occurred in a dog in group two, fourteen weeks after administration of sodium bromide. This dog's hemoglobin value gradually rose toward the control value by the nineteenth week. Two dogs in group one showed losses of approximately 2 Gm /100 ml of hemoglobin six weeks after administration of sodium bromide was begun. However, the anemias noted in most of the animals were not marked.

Leukocytosis.—Two of the dogs in group one showed striking increases in total leukocyte counts. These dogs showed counts of 83,000 and 53,000, four weeks after administration of sodium bromide was begun. One of them died at the end of the fifth week, the other continued to show an elevated leukocyte count at the seventh week and it died on the eighth week. Three of the dogs in group two developed leucocytosis of a milder degree but this decreased to approximately the control levels by the nineteenth week. There was little change in the differential leukocyte counts in any of the dogs and no early cell forms were seen.

Bone Marrow.—Studies were made of the bone marrow of the dogs in group 2. Twenty-eight examinations were made in all, both before and during administration of sodium bromide. The interpretation was based on inspection of all the material obtained; no differential counts were done. The Granulocytic/Erythrocytic ratios (G/E) of 3 dogs varied from 1/1 to 3/1. One dog persistently had ratios of 4/1 to 6/1. There was no significant change in cellularity or G/E ratio during the course of intoxication. No increases were noted in blast forms or defects in maturation. Megakaryocytes remained normal in number and appearance.

Sedimentation Rate.—Accelerated sedimentation rates were noted in all animals following administration of sodium bromide. These remained elevated throughout the course of intoxication. Such changes were seen even in those dogs which showed no signs

of anemia and so were probably not caused by changes in the size and hemoglobin content of the erythrocytes. These findings in dogs are in agreement with those of Dax (15) who reported accelerated sedimentation rates in 19 of 59 cases of human bromide intoxication. Since the sedimentation rate is influenced by the composition of the plasma proteins, acceleration of the rate may indicate that an imbalance in plasma protein fractions occurs during bromide intoxication.

SUMMARY

1. The progressive intoxication of dogs with sodium bromide results in a striking decrease in the volume of the extracellular space. In the terminal stages of intoxication, plasma volume may be decreased and serum sodium and potassium elevated as a result of hemoconcentration.

2. Both mild and transitory anemia and leucocytosis was observed in a number of dogs. Severe leukocytosis developed in only two dogs. Acceleration of the sedimentation rate occurred in most dogs during bromide intoxication.

REFERENCES

- (1) Moore, M., Soiler, T., and Alexander, L., *Confab Neuro*, 98, 1(1940)
- (2) Clark, B. B., Adams, W. L., and Morrissey, R., *J Pharmacol Exptl Therap*, 69, 280(1940)
- (3) Rosenblum, I., *ibid*, in press
- (4) Hawk, P. B., Oser, B. L., and Summerson W. H., "Practical Physiological Chemistry," 13th ed., Blakiston New York, 1954
- (5) Gomori, G., *J Lab Clin Med*, 27, 955(1941)
- (6) Gibson, J. G., and Evans, W. A. J., *J Clin Invest*, 17, 153(1938)
- (7) Gregerson, M. I., and Stewart, J. D., *Am J Physiol*, 125, 142(1939)
- (8) Bowler, R. G., *Biochem J*, 38, 385(1941)
- (9) Kibrich, A. C., Ross, M., and Rogers, H. F., *Proc Soc Exptl Biol Med*, 81, 353(1952)
- (10) Brode, B. B., and Friedman, M. M., *J Biol Chem*, 124, 511(1938)
- (11) Schwartz, S., Zieve, L., and Watson, C. J., *J Lab Clin Med*, 37, 843(1951)
- (12) Wintrobe, M. M., "Clinical Hematology," 11th ed. Lea and Febiger, Philadelphia, Pa., 1956, p. 314
- (13) Propp, S., *Blood*, 6, 585(1951)
- (14) Craven, E. B., *Am J Med Sci*, 186, 525(1933)
- (15) Dax, E. C., *Brit Med J*, 2, 226(1916)

Synthesis of Epoxide Polymers of Steroidal Compounds*

By W. F. HEAD, JR.,† and W. M. LAUTER

A series of twenty ethylene oxide and propylene oxide addition polymers of ursolic acid and some of its derivatives have been made and characterized. In addition, an ethylene oxide and propylene oxide polymer of cholic acid and ethylene oxide polymers of vitamin A, vitamin D₃, vitamin E, cortisone, estradiol, and testosterone are reported. Attempts to prepare steroidal polymers with highly branched or cross-linked side chains by using glycidol and epichlorohydrin were unsuccessful. The most acceptable structures as determined from molecular weight and solubility data were determined. Percentage polyalkylene oxide content, solubilities, and molecular weight or chain length distributions are presented.

URSOLIC ACID, its alkali, alkaline earth, and other metallic salts have been examined for possible use as emulsifiers in various emulsion, ointment, and cosmetic formulations (1, 2); however, none of these products offers serious competition to the presently available surface-active agents used for these purposes. The effectiveness of these salts is probably hindered by their extreme hydrophobic character. One of the aims of this investigation was to extend the work previously done in the light of some of the newer concepts of the chemistry of surface-active agents in the hope of obtaining a product or series of products derived from ursolic acid which are equal to or superior to those in present use.

The introduction of more hydrophilic groups into ursolic acid derivatives and similar compounds would seem to facilitate their surfactant properties by bringing about a better oil-soluble, water-soluble balance within the molecule. Hydrophilic, nonionic, polyalkylene oxide chains were utilized in this work because of their compatibility with a larger variety of materials. The number of these chains projecting from the steroidal nucleus can be varied by virtue of two reactive centers existing in ursolic acid. Consequently, the degree of hydrophilicity can be varied by the use of the proper intermediate. The intermediates were chosen so that the attachment of polyepoxide chains could be controlled in number and position, thus producing a series of compounds which would orient themselves

properly at various liquid-liquid interfaces. Also, two epoxide monomers, which produce polyalkylene oxide chains with different hydrophilicities, were used. These were ethylene oxide and propylene oxide, the latter being less hydrophilic. As this work progressed, the information obtained was extended to cholic acid, which is similar to ursolic acid in structure and properties, for purposes of increasing its own inherent surface activity.

Another facet of this investigation, that of inducing water solubility in a normally insoluble compound, was also suggested by the experimental data obtained using ursolic acid. A series of polyethylene oxide polymers of various water-insoluble vitamins and hormones were prepared with the hope of producing water-soluble, physiologically active materials. In this manner, pharmaceutical formulation and dosage administration problems might be minimized.

EXPERIMENTAL

Materials.—The monomers and initiators, with the exception of ursolic acid, were all obtained from reputable manufacturers and were considered to be of sufficient purity to use without further purification.

Generous quantities of crude ursolic acid were supplied by the National Cranberry Association, Hanson, Mass. The crude material contains 25–50% ursolic acid, the remainder being composed of other triterpenoids, resins, triterpenoid acids, sterols, waxes, extraneous plant material, and coloring matter. Purification was carried out by two methods, one employing decolorization of the ammonium salt (3) and the other simply decolorization of an alcoholic solution of the free acid (4). The former method is somewhat longer but purer ursolic acid is obtained after fractional crystallization. The semipure product from the free acid decolorization is obtained quickly and is probably 90–95% ursolic acid. Since the polymers produced are mixtures themselves, this material was also considered acceptable.

From the purified ursolic acid, various compounds were made so that polyepoxide chains could be

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attached selectively. These intermediates have been described previously and are as follows: ursonic acid (5), sodium ursolate (6), calcium ursolate (7), acetyl ursolyl amide (8-10), ursolyl amide (10), methyl ursolate (11), ursolyl amine C-2 (10), and ursolyl amine C-28 (10).

Methods.—For the preparation of these polymers, the reaction of liquified epoxides with initiator (corresponding to ursolic acid, its derivatives, cholic acid, and various vitamins and hormones) and catalyst under pressure and in the absence of solvent was utilized. A stainless steel bomb with snugly fitting, glass bomb liners of approximately 125-ml. capacity, E. H. Sargent and Co. number S-66950, was used. The top of the bomb was fitted with a pressure gauge reading 0-300 psig.

In general, 0.5 Gm. to 1.0 Gm. of initiator, approximately 0.5% to 1.0% catalyst, usually crushed sodium hydroxide or potassium hydroxide pellets, and 50 ml. to 60 ml. of epoxide at -20° were mixed well in the bomb liner, inserted into the bomb and heated. In some cases, the initiator was freely soluble in the monomer even at -20° ; it was felt that those initiators which were not completely soluble dissolved as the temperature was increased. The pellets of alkali catalysts were crushed to provide more catalytic surface area, although this meant the introduction of a very slightly larger quantity of moisture into the mixture. Moisture, in significant amounts, is very undesirable in these processes since epoxides react with water to produce polyepoxides at a rate highly competitive with the desired reaction. For this reason, all mixing was done as quickly as possible and the hygroscopic alkalis were not allowed to be exposed to the air any longer than necessary.

As the temperature increased, the pressure also increased and an attempt was made to find that temperature which would produce a pressure maximum of approximately 150 psig. In some cases, "peaking" occurred and pressures well in excess of 300 psig. were encountered. An indication of reaction termination was considered to be a drop in pressure without alteration of heat input. In some cases, with the more reactive initiators, a drop in pressure of 150 psi. took place within seven hours. With less reactive monomers or initiators a drop of only 10 psi. to 20 psi. was observed after forty-eight hours or even longer. This did not mean that polymeric material was not formed, rather that some monomer remained unreacted. Only a very small amount of epoxide is required to maintain a high pressure and rarely did pressures fall completely to 0 psig. without removing the source of heat.

At the termination of a reaction, excess unreacted monomer was allowed to escape from the safety valve and the bomb was opened. Products obtained were either waxy solids or semisolids or viscous, oily liquids. In most cases a slight tan coloration was imparted to the compounds and purification was considered necessary. For removal of this color, decolorization by heating a chloroform solution of the polymer mass with animal charcoal, filtration, and evaporation of the chloroform was employed.

It was considered necessary to determine if a polymer, other than a polyalkylene oxide, had been formed. This was accomplished by applying the

Liebermann-Burchard test for the presence of the steroidal nucleus to the purified polymer (12). In addition, certain solubility relationships were also used. While the ethylene oxide polymers produced were completely water soluble, a physical mixture of the polyethylene glycols, corresponding in molecular weight to that of the polymer formed, and the initiator material, when dissolved in water, deposited nearly all of the initiator in the form of a white precipitate. The propylene oxide polymers were insoluble in water; however, they did not deposit initiator material as did the corresponding control tests.

A series of reactions, using both ethylene oxide and propylene oxide, was performed, in the presence of catalyst only, for varying periods up to seventy-two hours. The amount of polymer formed was weighed. It is improbable that this polymer could have been formed from any reaction other than that between the epoxide and trace amounts of water in the catalyst, producing polyalkylene oxides, since initiators were not present. From these data, curves were constructed so that the amount of polyalkylene oxide in any of the steroidal polymer masses could be approximated by observing the time length of the reaction.

Polycapoxide chains terminate at some point in a hydroxyl group. The unknown factor in this series of compounds was the number of these chains projecting from a given initiator. This was usually determined by noting the number of hydrogen atoms, available for polymerization, appearing in the initiator. This is not always true because of steric hindrance, hydrolysis during the course of a reaction, etc.

The end hydroxyl groups of all polymers were determined by the method of Siggia (13). Calculations were then based on the most likely structure for determining the molecular weight. As additional data, molecular weights were also determined using an ebullioscopic method with chloroform as the solvent (14). If these two methods gave results which were in support of each other, the structure chosen was taken to be the most acceptable. If the physical data did not agree with the chemical method, the latter was recalculated using a different number of projected polyoxyalkylene chains. This changes the number of end group hydroxyls which, in turn, causes the molecular weight value to vary when the same data used before are applied. The number of chains were not simply picked at random, but had to be compatible with the number possible using a given initiator. Recalculations were made until the two methods were in agreement, then this structure was accepted. In some cases, the polymers were polyoxyalkylene esters. Therefore, for these compounds, a third molecular weight determination was made using saponification data (15). Agreement for one structure was then required of all three methods of analysis. In this way, the most acceptable structure and molecular weight for all polymers were determined.

In order to determine the molecular weight distribution and the corresponding percentages by weight of the total polymer mass, a Poisson distribution, based on the determined average molecular weight, was calculated (16). The average number of epoxide units per polymer molecule, as

obtained from molecular weight data, is represented by ν in the equation below, where W_x is the weight percentage of a particular X-mer, and X is the number of epoxide units in this X-mer.

$$W_x = \left[\frac{\nu}{\nu + 1} \right] \frac{X^{\nu X - 2}}{e^{\nu} (X - 1)!}$$

A representative curve is shown in Fig 1 for the ethylene oxide polymer of ursolic acid. The values of ν for the remainder of the polymers are reported as average chain length in Tables I and II.

Results.—The most acceptable structures for the polymers reported are the same as would be predicted from the active polymerizable hydrogen content of the respective initiators, with the exception of the ethylene oxide polymer of acetyl ursolyl amide and the propylene oxide polymer of ursolic acid.

Since the acetyl group has active hydrogen atoms, it is possible that epoxide reaction could occur at this point. In order to determine if this mechanism was taking place in the synthesis of the acetyl ursolyl amide-ethylene oxide polymer, pregnenolone acetate, a compound containing a ketonic carbonyl group and an acetate ester but no other source of available hydrogen, was chosen as a blank. A poly-

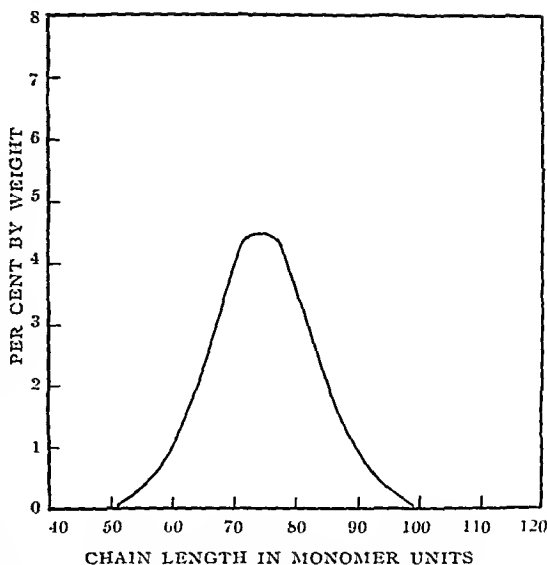


Fig 1—Distribution of chain length in ursolic acid-ethylene oxide polymer.

TABLE I.—REACTION CONDITIONS AND PROPERTIES OF ETHYLENE OXIDE POLYMERS

Initiator	Reaction		Polyethylene- Oxide Content, %	Molecular Weight ^a			Av. Chain Length ^b
	Time, Hr	Temp, °C		A	B	C	
Ursolic acid	7	100	8.0	3,657	3,360	4,793	73
Ursolic acid	12	100	3.9	1,700	2,170	1,485	28
Sodium ursolate	14	100	6.5	1,260	1,400		18
Calcium ursolate	20	100	10.0	2,492	2,100		35
Methyl ursolate	50	100	53.5	707	885		5
Acetyl ursolyl amide	48	110	59.0	890	1,200	2,200	5 ^c
Ursolyl amide	48	110	50.0	9,150	8,500		197
Ursolyl amine C-28	62	110	31.4	2,220	1,950		40
Ursolyl amine C-2	50	110	38.4	7,000	6,500		149
Cholic acid	46	110	30.4	9,150	9,800		164
Crude ursolic acid	67	110	27.0	5,000	6,300		103
Vitamin A	20	100	10.0	1,600			30
Vitamin E	10	100	3.7	780			8
Vitamin D ₂	16	100	7.0	2,760			54
Cortisone	20	100	10.0	1,514			26
Estradiol	11	100	3.7	2,000			39
Testosterone	13	100	6.0	830			12

^a A—End group hydroxyl determination, B—ebullioscopic data, and C—saponification data.

^b Reported as monomer units per polymer molecule, based on end group hydroxyl determination.

^c For discussion see results.

TABLE II.—REACTION CONDITIONS AND PROPERTIES OF PROPYLENE OXIDE POLYMERS

Initiator	Reaction		Polypropylene- Oxide Content, %	Molecular Weight ^a			Av. Chain Length ^b
	Time, Hr	Temp, °C		A	B	C	
Ursolic acid	30	110	8.3	1,015	935	770	10
Ursolic acid	48	135	17.7	1,050	900	727	11
Sodium ursolate	21	135	5.6	835	1,000		6
Calcium ursolate	46	135	16.9	1,910	2,200		17
Methyl ursolate	72	135	49.0	719	1,000		4
Acetyl ursolyl amide	49	135	62.0	1,425	1,400	1,540	16
Ursolyl amide	60	135	45.0	3,882	3,600		59
Ursolyl amine C-28	62	135	73.0	2,600	2,200		37
Ursolyl amine C-2	72	135	69.0	3,094	2,750		53
Cholic acid	46	135	19.5	5,800	5,600	5,010	93
Crude ursolic acid	49	135	29.0	1,184	1,310		13

^a A—End group hydroxyl determination, B—ebullioscopic data, and C—saponification data.

^b Reported as monomer units per polymer molecule, based on end group hydroxyl determination.

merization reaction similar to the general method was carried out using this compound as the initiator and sodium hydroxide as the catalyst. After only forty eight hours at 110° (compared to fifty eight hours for the acetyl ursolic amide initiated reaction), a polymeric mass was obtained. Attempts were made to isolate pregnenolone acetate from the mixture by Soxhlet extraction using hexane and by preparing the semicarbazone from an aqueous solution, however, these attempts failed. It was concluded, therefore, that pregnenolone acetate had, by some mechanism, become incorporated as a chemical part of the polymer molecule.

It is possible that ethylene oxide reacted with the active hydrogen of the acetyl group or that from the ketone group. Hydrolysis also might have occurred and left a hydroxyl group in the place of the acetyl group. A mechanism similar to the first is known to take place with the carbamion of ethyl acetoacetate (17).

As noted in Table I, the average molecular weight values, as determined by three methods are not close enough to assign any one structure to the polymer and it is probable that a mixture of materials was obtained. Saponification and hydroxyl value molecular weight are based on a polymer whose initiator contains an intact, unsubstituted acetyl group and two polyoxyethylene chains attached to the amide nitrogen.

The propylene oxide polymer of this same initiator does not show this apparent reaction course. If the acetyl group remains intact and carbamion formation assumes secondary importance, then the only locus of reaction is the sterically hindered amide function. The low yield obtained of this polymer might be explained on this basis. Additional supporting evidence was the failure of pregnenolone acetate initiated reactions to produce any polymeric material with the less reactive propylene oxide.

The propylene oxide polymer of ursolic acid appears to have only one polymer side chain attached to the nucleus. The analogous polymer in the ethylene oxide series has long chains attached to both the carboxyl and hydroxyl groups of ursolic acid, while the propylene oxide polymer seems to contain a hydroxyl chain only. A structure which contains one terminal hydroxyl group is the only one which will correlate the analytical data. A carboxylate polymer would not be justifiable, since the secondary hydroxyl group on the original ursolic acid nucleus would respond to acetylation (as performed in the analysis for hydroxyl value) as would the hydroxyl group of the polymer chain. The steric hindrance imposed on the carboxyl group by the methyl groups in positions 14 and 20, in addition to the more bulky nature of propylene oxide as compared to ethylene oxide, are probably factors which cause hydroxyl reaction to be preferable.

Since the polymers of vitamins A, D, and E, and estradiol, cortisone, and testosterone were prepared primarily to see if they were water soluble, only the ethylene oxide series was prepared. All of these

polymers were water soluble, vitamin A polymer solutions being opaque. Whether the polymers have retained the pharmacologic activity of the respective initiator is being investigated.

The specific properties of each polymer and reaction conditions are summarized in Tables I and II. Generally, the ethylene oxide polymers were either solids or semisolids, melting from 27° to 52°, and being soluble in water and chloroform and slightly soluble to insoluble in organic solvents such as acetone, alcohol, benzene, hexane, and ether. The propylene oxide polymers were all viscous liquids and were soluble in most organic solvents and insoluble in water.

Polyepichlorohydrin contains a regularly repeating chloromethyl group which may be reacted with magnesium oxide to produce an ether linkage between individual chains (18). If ursolic acid or its derivatives could be used to initiate the polymerization, it too would appear at intervals in this cross-linked polymer. In order to investigate the properties of a polymer whose side chains occupy a comparatively large area with respect to ursolic acid, attempts were made to prepare these compounds. Various heating times, temperatures up to 200°, and sodium hydroxide, aluminum chloride, metallic sodium, and aqueous and concentrated sulfuric acid as catalysts were employed but the desired products did not materialize.

With the same purpose in mind, glycidol was used as the monomer. When this epoxide attaches to an initiator, a secondary alcohol group is generated in addition to the primary hydroxyl group already present. Again, acceptable products were not obtained. It is thought that monomer self-polymerization is a much more predominant reaction than the reaction with ursolic acid.

REFERENCES

- (1) Eisen, H., and Skauen, D. M., *Drug Standards* 23, 186(1955).
- (2) Kruekman, A., *Am. Perfumer Essent. Oil Rev.*, 42, 31(March 1941).
- (3) Wu, B. Y. T., and Parks, L. M., *THIS JOURNAL* 42, 602(1953).
- (4) Eisen, H., *The Preparation and Physicochemical Studies of the Metallic Salts of Ursolic Acid*, University Microfilms Inc., Ann Arbor, Mich., 1955.
- (5) Jacobs, W. A., and Fleck, C. E., *J. Biol. Chem.* 92, 478(1931).
- (6) Sando, C. E., *ibid.* 56, 463(1923).
- (7) Sell, H. M., and Kremers, R. E., *Ind. Eng. Chem. Anal. Ed.* 7, 105(1935).
- (8) Sando, C. E., *op. cit.* 90, 177(1931).
- (9) Sell, H. M., and Kremers, R. E., *J. Biol. Chem.* 125, 451(1938).
- (10) Wu, B. Y. T., and Parks, L. M., *op. cit.* 42, 603(1953).
- (11) Orr, J. E., *et al.*, *THIS JOURNAL* 34, 39(1945).
- (12) Gardner, J. A., *Allen's Commercial Organic Analysis*, Vol. II, 5th ed., The Blakiston Co., Inc., Philadelphia 1924, p. 768.
- (13) Siggia, S., *Quantitative Organic Analysis via Functional Groups*, John Wiley and Sons, Inc., New York, 1919, p. 4.
- (14) Cheronis, N. D., *Technique of Organic Chemistry*, Vol. VI, Edited by A. Weissberger, Interscience Publishers, Inc., New York, 1954, p. 216.
- (15) Siggia, S., *op. cit.*, p. 24.
- (16) Flory, P. J., *J. Am. Chem. Soc.* 62, 1561(1940).
- (17) Johnson, W. L., U. S. pat. 2,143,827, June 22, 1918.
- (18) Flory, P. J., *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, N. Y., 1953, p. 356.

Surface Activity of Ethylene Oxide and Propylene Oxide Polymers of Steroidal Compounds*

By W. F. HEAD, Jr.,† and W. M. LAUTER

The effects of the ethylene oxide polymers of ursolic acid, some of its derivatives, and cholic acid on surface and interfacial tensions and on the formation of oil-in-water emulsions and transparent emulsions are described. In addition, the cotton tape modification of the Draves test was employed for evaluation of these compounds as wetting agents. The propylene oxide polymers of these same initiators were found to be incapable of aiding in the formation of water-in-oil or oil-in-water emulsions. These polymers were evaluated by preparing a series of water-in-oil ointments and by noting their effects on interfacial tension.

IN THE PRECEDING PAPER, the preparation of a series of polymers, designed in structure to fulfill the requirements of surface-active agents, was described. To evaluate these polymers as surfactants, the usual methods were employed using rather widely accepted commercial products as standards of comparison

EXPERIMENTAL

Surface Tension and Interfacial Tension Measurements.—Perhaps the most common evaluation technique used for any surfactant is to measure its ability to decrease the surface tension exerted by water. One-tenth per cent aqueous solutions of the polymers in the ethylene oxide series were screened for this ability using the du Nouy tensiometer method. After these data were collected, the solutions were overlaid with light liquid petrolatum and the interfacial tensions determined. Since the polymers in the propylene oxide series were all insoluble in water, data were collected for interfacial tensions only. This was accomplished in the same manner as with the ethylene oxide polymers except that the propylene oxide polymers, 0.1%, were dissolved in the light liquid petrolatum, then underlaid with water.

For comparison purposes, data were obtained for 0.1% solutions of Tween 80® (polyoxyethylene sorbitan monooleate)¹ and Aerosol O T² (dioctyl sodium sulfosuccinate) along with the ethylene oxide polymers. As the standard of comparison for the propylene oxide polymers, a 0.1% solution of Span 60® (sorbitan monostearate)¹ in light liquid petrolatum was employed. Polyethylene glycol and polypropylene glycol solutions were tested in a similar manner in order to see if the steroidal polymers were more surface active. The results are shown in Table I.

Wetting Agent Evaluation.—The capability of a surfactant to be a good wetting agent is frequently

TABLE I—SURFACE TENSION EFFECTS OF VARIOUS POLYMERS AND CERTAIN STANDARDS^a

Solution	Surface Tension, Dynes/cm	Interfacial Tension in Dynes/cm
Distilled Water	72.0	46.3
Tween 80	42.5	7.8
Aerosol O T	30.8	2.5
Polyethylene Glycol 1540	62.5	31.9
Polyethylene Glycol 4000	62.5	29.9
Ethylene Oxide Polymer of:		
Ursolic Acid	44.1	9.7
Ursonic Acid	42.3	7.8
Sodium Ursolate	43.7	8.6
Calcium Ursolate	42.8	8.2
Methyl Ursolate	43.3	10.6
Acetyl Ursolyl Amide	41.5	9.5
Ursolyl Amide	43.3	9.4
Ursolyl Amine C-28	43.0	12.5
Ursolyl Amine C-2	49.3	17.5
Cholic Acid	52.8	19.4
Crude Ursolic Acid	49.9	18.0
Span 60		3.1
Polypropylene Glycol 425		25.5
Propylene Oxide Polymer of:		
Ursolic Acid		10.3
Ursonic Acid		10.5
Sodium Ursolate		11.7
Calcium Ursolate		14.0
Methyl Ursolate		9.8
Acetyl Ursolyl Amide		8.5
Ursolyl Amide		8.4
Ursolyl Amine C-28		9.9
Ursolyl Amine C-2		8.0
Cholic Acid		8.2
Crude Ursolic Acid		10.0

^a Data presented for solutions of 0.1% solute concentration.

tested by the Draves method. The principle of this test is the comparison of times required for the complete wetting and sinking of a skein of yarn by solutions of the test material and some standard. Since a large number of readings are required for accuracy, various modifications have been suggested. The cotton tape modification of the Draves test was chosen to be used in this work (1). Rather than using skeins of yarn which contain considerable entrapped air, special cotton tapes of uniform specifications were used. Two tape tests are adequate to give accuracy within 5%, while thirteen skein tests are required to give the same degree of accuracy. Since only the ethylene oxide polymers are

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¹ Atlas Powder Company, Wilmington, Del.
² American Cyanamide and Chemical Corp., New York, N. Y.

water soluble, this test could not be applied to the propylene oxide polymers. One-tenth per cent solutions of the comparison standards and the various polymers were used and their respective wetting times taken at a temperature of 33°. The results are shown in Table II.

Emulsion Tests.—The ethylene oxide polymers, which were designed for oil-in-water emulsification, were used in a series of emulsions and compared with Tween 80. Several emulsion formulations, varying

in percentage composition of ingredients, were initially tested. The emulsions containing 40% mineral oil, 5-7% polymer, and 53-55% water were made by mixing in a Waring Blendor for one minute. These emulsions were much too well formed to allow any significant differential comparison.

The formula finally selected for comparison consisted of 70% water, 28% mineral oil, and 2% emulsifier. These ingredients were mixed in a Waring Blendor for ten seconds; the emulsions formed were then passed twice through a hand homogenizer. The emulsions were evaluated immediately after preparation and after two weeks storage at 45°. This storage period is equivalent to approximately six months at room temperature (2). The general appearance, relative viscosity at 31°, approximate particle size as obtained using an ocular micrometer, and globule shapes are recorded in Tables III and IV.

Ointment Tests.—Several attempts were made at preparing 30% oil-in-water and water-in-oil emulsions using 2-4% Span 60, Span 80®,¹ and the propylene oxide polymer of ursolic acid as the emulsifying agents. The ingredients were mixed for one minute in a Waring Blendor, then passed through a hand homogenizer three times. None of these emulsions were good; complete splitting of phases took place within five minutes.

For gross comparative purposes, a water-in-oil ointment formulation was selected. The oil phase portion of the formula consisted of 10% emulsifier, 5% beeswax, 60% petrolatum, and 25% mineral oil (3). This mixture was heated to 65° and 30%

TABLE II.—WETTING TIMES OF ETHYLENE OXIDE POLYMERS AND CERTAIN STANDARDS^a

Solution	Av. Wetting Time, Sec.
Distilled Water	108.6
Tween 80	22.5
Aerosol O T	1.1
Polyethylene Glycol 4000	42.7
Ethylene Oxide Polymer of:	
Ursolic Acid	33.5
Ursonic Acid	26.5
Sodium Ursolate	19.6
Calcium Ursolate	8.3
Methyl Ursolate	31.3
Acetyl Ursolyl Amide	27.9
Ursolyl Amide	57.0
Ursolyl Amine C-28	54.3
Ursolyl Amine C-2	63.2
Cholic Acid	90.7
Crude Ursolic Acid	57.2

^a Data presented for solutions of 0.1% solute concentration.

TABLE III.—CHARACTERISTICS OF EMULSIONS MADE USING ETHYLENE OXIDE POLYMERS IMMEDIATELY AFTER PREPARATION^a

Emulsifier	General Appearance ^b	Relative Viscosity ^c	Approximate Particle Size %	Distribution μ	Globule Shape
Tween 80	Good	1.90	95-5	1-5	Round
Ethylene Oxide Polymer of:					
Ursolic Acid	Good	1.81	90-10	1-10	Round
Ursonic Acid	Good	2.46	75-25	1-2.5	Round
Sodium Ursolate	Good	2.24	95-5	1-2.5	Round
Calcium Ursolate	Good	2.28	90-10	1-5	Round
Methyl Ursolate	Good	1.77	50-50	1-35	Very distorted
Acetyl Ursolyl Amide	Good	3.34	95-5	1-4	Round
Ursolyl Amide	Good	2.01	95-5	1-1.5	Round
Ursolyl Amine C-28	Good	1.98	95-5	1-1.5	Round
Ursolyl Amine C-2	Good	2.73	95-5	1-2	Round
Cholic Acid	Good	2.50	90-10	1-2.5	Round
Crude Ursolic Acid	Good	3.58	50-50	1-8	Slightly distorted

^a Attempts to make an emulsion using 2% ursolic acid as the emulsifier completely failed, even when the mixing time in the Waring Blendor was increased to one minute.
^b All of the emulsions seemed to begin creaming within one hour after their preparation. On shaking, uniformity was readily obtained. The emulsions were all white and creamy with the exception of the Tween 80 and the acetyl ursolyl amide polymer emulsions which had a very faint yellow tint.
^c Determined at 31° using an Ostwald viscosimeter.

TABLE IV.—CHARACTERISTICS OF EMULSIONS MADE USING ETHYLENE OXIDE POLYMERS AFTER TWO WEEKS STORAGE AT 45°

Emulsifier	General Appearance	Relative Viscosity ^a	Approximate Particle Size Distribution μ		Globule Shape
			%	μ	
Tween 80	Good	2.08	95 5	1 5	Round
Ethylene Oxide Polymer of:					
Ursolic Acid	Some oil-water separation	2.28	85 15	1 10	1% distorted
Ursonic Acid	Very slight oil-water separation	1.78	75 25	1 2.5	Round
Sodium Ursolate	Some oil-water separation	2.31	95 5	1 2.5	Round
Calcium Ursolate	Very good	1.97	90 10	1 5	Round
Methyl Ursolate	Poor	2.12	80 20	2.5 20	Round
Acetyl Ursolyl Amide	Very poor	1.75	80 20	1.5 10	20% distorted
Ursolyl Amide	Poor	1.86	90 10	1 5	5% distorted
Ursolyl Amine C-28	Completely split	1.38	80 20	1.5 10	1% distorted
Ursolyl Amine C-2	Very slight oil-water separation	2.52	95 5	1 10	5% distorted
Cholic Acid	Good	2.55	90 10	1 2.5	Round
Crude Ursolic Acid	Very slight oil-water separation	3.43	50 50	1 10	Round

^a Determined at 31° using an Ostwald viscosimeter.

of its weight in water, at 70°, was added with continuous stirring until the ointment was cool. The necessary amounts were chosen in order to prepare two ounces of ointment with each emulsifier. As the comparison standard emulsifier, Arlacel 83[®] (sorbitan sesquioleate) was used. Some of the ointments besed as much as 60% initially. In these cases, the excess water was poured off and the ointment stored at 45° to see if further bleeding would occur. The general description, bleeding characteristics, and comparative ranks of these ointments, immediately after preparation and after two weeks storage at 45°, are recorded in Table V and Table VI.

Transparent Emulsions.—Three of the polymers prepared in the ethylene oxide series had calculated

TABLE VI.—OINTMENT CHARACTERISTICS AFTER TWO WEEKS STORAGE AT 45°

Emulsifier	Description	Bleeding	Rank
Arlacel 83	Good	Very slight	2
Propylene Oxide Polymer of:			
Ursolic Acid	Very poor	9%	7
Ursonic Acid	Fair	1%	4
Sodium Ursolate	Fair	1%	4
Calcium Ursolate	Fair	1%	4
Methyl Ursolate	Poor	4%	5
Acetyl Ursolyl Amide	Very good	None	1
Ursolyl Amide	Very poor	8%	6
Ursolyl Amine C-28	Fair	1%	4
Ursolyl Amine C-2	Fair	1%	4
Cholic Acid	Very poor	9%	7
Crude Ursolic Acid	Very poor	9%	7
None	Good	Very slight	3

TABLE V.—OINTMENT CHARACTERISTICS IMMEDIATELY AFTER PREPARATION

Emulsifier	Description	Bleeding	Rank
Arlacel 83	Excellent	None	1
Propylene Oxide Polymer of:			
Ursolic Acid	Very poor	50%	6
Ursonic Acid	Very poor	60%	5
Sodium Ursolate	Fair	10%	4
Calcium Ursolate	Poor	20%	5
Methyl Ursolate	Good	Very slight	4
Acetyl Ursolyl Amide	Very good	None	2
Ursolyl Amide	Poor	25%	6
Ursolyl Amine C-28	Very poor	40%	6
Ursolyl Amine C-2	Poor	25%	7
Cholic Acid	Good	Very slight	4
Crude Ursolic Acid	Fair	20%	4
None	Good	Very slight	3

HLB values (3) which indicated that they might possibly be utilized in the preparation of transparent emulsions. The polymers involved were those initiated by ursolic acid, cholic acid, and crude ursolic acid.

A typical formula consisting of 1% spearmint oil, 15% emulsifier, and 84% water was selected (3). The polymers were dissolved in the oil and water was added slowly with vigorous stirring.

Thin, white, nontransparent emulsions were formed with all three polymers. After twenty-four hours, the emulsions were still well formed but not transparent. These results were not entirely un-

expected since the HLB values are merely indicative of possible application.

DISCUSSION OF RESULTS

The ursonic acid-ethylene oxide polymer had the most profound effect on interfacial tension. This tension was 7.8 dynes per centimeter. The lowering of surface tension was in the range of 30 dynes per centimeter with all ethylene oxide polymers. These results are very favorable and comparable to the standards used.

The calcium ursolate-ethylene oxide polymer was, by far, the best wetting agent of the series, although it did not approximate Aerosol O T. It was, however, much better than Tween 80.

On the basis of general appearance, approximate particle size distribution, and storage tests, the calcium ursolate-ethylene oxide polymer also produced the best oil-in-water emulsion. Good emulsions were also obtained using the polymers initiated by ursonic acid, ursolyl amine C-2, cholic acid, and crude ursolic acid.

The ethylene oxide polymers of ursolic acid, cholic acid, and crude ursolic acid had calculated HLB values which indicated that they might be capable of producing transparent emulsions. On preparation of some of these emulsions, this property was found to be lacking. It should be pointed out that these compounds are atypical with respect to the types of substances for which the HLB value calculations were set up.

Of the propylene oxide polymers, those initiated by acetyl ursolyl amide, ursolyl amide, ursolyl amine C-2, and cholic acid were best in the ability

to lower interfacial tension. They were not, however, as effective as Span 60 which was the standard of comparison.

In general, the ointments produced using the propylene oxide polymers as the emulsifier were very poor in comparison with the standard. Only one polymer, the acetyl ursolyl amide-propylene oxide polymer, was effective. Initially, the ointment produced by this polymer was slightly inferior to the standard; however, it was more stable and after two weeks storage at 45° was the best ointment in the series.

On the basis of these results, it is believed that some of the ethylene oxide polymers have properties which would make them comparable with other surfactants on the market. If the purification procedure used for ursolic acid could be omitted and the crude product used, commercial production would be simple and inexpensive. Because of the expense involved, it is also important that, if possible, the preparation of derivatives be avoided, even though some of the polymers of these initiators were more effective. Since the calcium ursolate-ethylene oxide polymer, showed the most promise, it might be advisable to attempt the preparation of this initiator salt from crude ursolic acid.

The propylene oxide polymers do not seem to have sufficient merit to warrant further consideration.

REFERENCES

- (1) Shapiro, L., *Am. Dyestuff Repr.*, **39**, 38(1950).
- (2) Lauter, W. M., private communication.
- (3) Atlas Powder Company, "A Guide to Cosmetic and Pharmaceutical Formulation with Atlas Products," Wilmington, Del., 1953.

A Polarographic Study of β -Haloethylamine Adrenergic Blocking Agents*

By ROGER MANTSAVINOS† and JOHN E. CHRISTIAN

A polarographic procedure is described for measuring the relative rate of cyclization of the β -haloethyl group of Dibenamine-like compounds to the ethyleneimonium form. This method is based on the rapid reaction of thiosulfate with ethyleneimonium ions. The thiosulfate reaction product of several compounds has been found to be polarographically reducible and with Dibenzylamine and Dibenamine the heights of the polarographic waves were found to be proportional to various concentrations of the reducible species. The polarographic waves appear to be irreversible and a two electron reduction has been postulated.

ETHYLENEIMONIUM IONS formed by the cyclization of β -haloethyl groups of Dibenamine-like compounds are believed to be responsible for the adrenergic blocking action of these compounds (1). Although ethyleneimonium ions in solutions of aliphatic β -chloroethyl-

amines (nitrogen mustards) are polarographically reducible (2), ethyleneimonium ions formed by the Dibenamine series of compounds are relatively unstable in solution (3) and do not appear to be reducible at the dropping mercury electrode (D.M.E.). No reducible species could be detected in 60 per cent ethanol solutions of Dibenamine or Dibenzylamine buffered with acetate at an apparent pH of 7.0. However, the reaction

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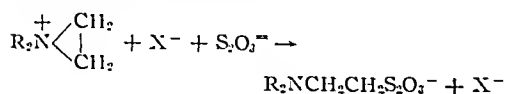
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product of several ethyleneimonium intermediates, of the Dibenamine type, with thiosulfate were found to be polarographically reducible. Based on this thiosulfate reaction, polarographic procedures were developed for measuring the relative rate of cyclization of β -haloethylamine adrenergic blocking agents and for determining the amount of thiosulfate reaction product formed. It is of interest to note that although ethyleneimonium forms of Dibenamine-like compounds are not polarographically reducible, Lordi and Christian (4), in a physicochemical study of antihistamines, found ethyleneimonium ions of N-ethyl-N-1-naphthylmethyl-2-chloroethylamine (SY-14) and its thiosulfate reaction product to be polarographically reducible in $M/15$ phosphate buffer, pH 5.9.

EXPERIMENTAL

Materials and Apparatus.—A Sargent Model XXI visible recording polarograph with a D.M.E. and a Lingane-Laitinen H-type electrolysis vessel (5) containing a saturated calomel electrode (S.C.E.) was used for most polarographic work. For kinetic experiments a Carritt type H-cell (6) was used to prevent chloride ion contamination from the salt bridge. Prior to recording polarograms, test solutions were degassed for twenty minutes with tank nitrogen which was purified by passage through a train of vanadous sulfate solution (7) and distilled water. The nitrogen was then saturated with the solvent of the test solution before degassing the test solution. Triton X-100, 0.002% was used as maximum suppressor. The capillary used throughout these studies had a constant $m^{2/3} + 1/6$ value of $1.853 \text{ mg.}^{2/3} \text{ sec.}^{-1/2}$ in the base solution at -0.25 volt vs. S.C.E., under a pressure of 60 cm. of mercury. The base solution, having an apparent pH of 7.0, was made by diluting 20 ml. of an acetate buffer concentrate consisting of 18.8 ml. of 1.0 N sodium acetate and 1.2 ml. of 1.0 N acetic acid prepared in 60% ethanol. The acetate buffer also served as supporting electrolyte. Analytical reagent grade chemicals were used throughout. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) was first treated to remove extraneous water (8) before being used to prepare standard stock solutions in 60% ethanol. Experimental samples of β -haloethylamines were generously supplied by the Smith, Kline and French Research Laboratories, Philadelphia. Due to the limited aqueous solubility of these compounds a solvent of 60% ethanol was used in all experiments.

Kinetic Studies.—Whenever thiosulfate is added to a solution containing ethyleneimonium ions, an instantaneous reaction occurs resulting in the formation of an ethyl thiosulfate derivative (3) as illustrated by the following reaction:



Since this reaction occurs instantaneously, the rate

of thiosulfate consumption becomes an indirect measure of the relative rate of cyclization of a β -haloethylamine into the physiologically active ethyleneimonium ion (3). By polarographically following the change in concentration of thiosulfate with time, the relative rate of cyclization of certain β -haloethylamines was determined. The following procedure was used to measure the rate of cyclization for a number of compounds. A solution containing 20 ml. of acetate buffer concentrate, 1.0 ml. of 0.2% Triton X-100, and a known amount of $1.80 \times 10^{-2} M$ sodium thiosulfate (in excess of the amount of amine to be used) was degassed for thirty minutes in a flask immersed in a water bath maintained at $25^\circ \pm 0.5^\circ$. A second solution of 60% ethanol was simultaneously degassed under similar conditions. After the solutions were deoxygenated an accurately weighed quantity of β -haloethylamine was dissolved in 50 ml. of degassed ethanol contained in a 100-ml. volumetric flask. To this solution was added the acetate buffer-thiosulfate solution and the resulting solution diluted to 100 ml. with degassed ethanol. The final solution was mixed, and an adequate portion was placed in the solution compartment of a Carritt type H-cell and degassed for a final few seconds. At an arbitrarily chosen zero time, a constant potential of -0.025 volt vs. S.C.E. was applied and readings of current were made as a function of time. The impressed voltage corresponded to a point on the plateau of the thiosulfate polarographic wave. When the reaction was essentially complete, as evidenced by a steady current, the chart paper containing the current-time curve was removed and the current readings corrected for residual current before graphically determining the velocity constants. In all cases examined the cyclization process was found to be first order over the time period studied (not over thirty minutes for any given experiment). The accepted velocity constant, k , represents an average of three values of the slopes of curves constructed by plotting the natural log of the limiting current against time. A straight line was drawn through the experimental points by inspection (9). The presence of mercury in the reaction mixture did not influence the progress of the reaction. Table I contains experimental data from which the rate of cyclization of Dibenzylamine was calculated. Velocity constants of $0.00928 \text{ min.}^{-1}$ for Dibenzylamine, 0.0144 min.^{-1} for N-(α -naphthylmethyl)-N-ethyl- β -chloroethylamine and $0.00525 \text{ min.}^{-1}$ for 1-dibenzylamino-2-chloropropane were obtained when determined by this same method. The above procedure is not applicable to compounds such as certain bromo analogs which cyclize at an extremely rapid rate nor to iodo compounds which interfere with the recording of the thiosulfate wave.

Polarographic Determination of the Thiosulfate Reaction Product.—The ethyleneimonium-thiosulfate reaction product of certain β -haloethylamines, henceforth referred to as ET, gave a well-defined polarographic wave upon reduction at the D.M.E. Since anodic thiosulfate waves are obtained at more positive potentials than cathodic ET waves, both residual thiosulfate and ET can be determined polarographically in the same solution. The following procedure was used for the polarographic determination of the thiosulfate reaction product. An accurately weighed amount of the amine hydro-

TABLE I.—CYCLIZATION OF DIBENZYLINE IN ETHANOLIC THIOSULFATE SOLUTIONS, 25°

Dibenzyl- line HCl→ Thiosulfate→ Time (t) (Min.)	Initial Concentration of Reactants			
	1.00 × 10 ⁻³ M 1.80 × 10 ⁻³ M	1.50 × 10 ⁻³ M 2.70 × 10 ⁻³ M	2.00 × 10 ⁻³ M 2.70 × 10 ⁻³ M	
	<i>i</i> _t ^a (Microamp.)	<i>i</i> _t ^a (Microamp.)	<i>i</i> _t ^a (Microamp.)	
0	3.84	7.68	5.18	
5	3.32	6.72	4.44	
10	2.96	5.82	4.00	
15	2.76	5.28	3.60	
20	2.44	4.89	3.32	
	<i>k</i> ^b = 0.0212 min. ⁻¹	<i>k</i> ^b = 0.0217 min. ⁻¹	<i>k</i> ^b = 0.0215 min. ⁻¹	
		Mean <i>k</i> value.....	0.02146 min. ⁻¹	

^a Value of the diffusion current of thiosulfate at time *t*.
^b *k* values as determined graphically from the slope of a plot of ln *i*_t versus time.

chloride, sufficient to prepare 100 ml. of a 1.00 × 10⁻³ M to 4.00 × 10⁻³ M solution, was dissolved in 50 ml of 60% ethanol contained in a 100-ml. volumetric flask. After the amine was solubilized, 20 ml. of acetate buffer concentrate, 1.0 ml. of 0.2% Triton X-100, and a known excess of a fresh stock solution of 2.00 × 10⁻² M sodium thiosulfate were added to the solution. The resulting solution was diluted to 100 ml. with 60% ethanol, immersed in a constant temperature bath maintained at 25° ± 0.5°, and the reaction allowed to run to completion. The reaction was considered to be complete when a constant diffusion current was obtained by electrolyzing a degassed volume of the test solution from approximately -0.60 to -1.5 volt *vs.* S.C.E. On completion of the reaction, polarograms of degassed test solutions were recorded over this same potential span. A blank solution containing the same volume of thiosulfate as that contained in the standard solution was also run. The amount of thiosulfate consumed was determined by subtracting the diffusion current of the residual thiosulfate in the test solution from the diffusion current of the thiosulfate in the blank solution, and then determining the equivalent concentration from a previously constructed wave height-concentration curve of thiosulfate. This thiosulfate curve was constructed from data obtained under the same conditions at which thiosulfate was made to react with the amine. The amount of thiosulfate consumed in the reaction was taken to be equivalent to the amount of ET in solution. Table II presents experimental data demonstrating the proportionality between the diffusion current obtained by the reduction of ET, and both the concentration of the reducible species and the original concentration of amine. In addition to Dibenzyl- and Dibenamine it was of interest to note whether other β-haloethylamines would form reducible ET derivatives, and if the shapes of the reduction waves were suitable for polarographic analysis. The results of such studies are presented in Table III. The experimental data of Table III were obtained by electrolyzing a solution containing an excess of thiosulfate and the hydrochloride of the compound listed. In all cases in which well-defined polarographic waves were obtained the *E*_{1/2} values were found to shift to more negative potentials with an increase in concentration. The physiologically inactive compound, dibenzylaminoethanol, did not form a reducible thiosulfate substitution product.

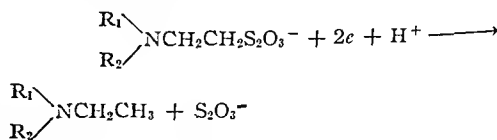
Postulated Mechanism of Reduction.—A first

TABLE II.—CALIBRATION DATA FOR DETERMINING ET FORMED IN THE REACTION OF THIOSULFATE WITH DIBENZYLINE AND DIBENAMINE

Diben- zyl- line HCl (mM) Liter ⁻¹)	Thiosul- fate ^a Con- sumed (mM) Liter ⁻¹)	<i>i</i> _d (ET) (Micro- amp.)	<i>E</i> _{1/2} <i>vs.</i> S.C.E. (Volts)	<i>i</i> _d / <i>C</i>
Dibenzyl- line				
1.00	1.00	3.66	-1.06	3.66
1.50	1.51	5.28	-1.06	3.50
2.00	2.10	7.20	-1.07	3.43
2.50	2.48	9.12	-1.07	3.68
3.00	3.00	11.2	-1.08	3.73
3.50	3.51	13.0	-1.09	3.71
4.00	3.98	14.6	-1.09	3.67
Diben- amine				
HCl (mM) liter ⁻¹)				
Dibenamine				
1.00	1.02	4.38	-1.06	4.29
1.50	1.52	6.48	-1.06	4.26
2.00	2.00	8.55	-1.06	4.28
2.50	2.47	10.9	-1.07	4.41
3.00	2.93	13.2	-1.07	4.51
3.50	3.47	15.3	-1.07	4.38
4.00	3.95	17.6	-1.08	4.46

^a This value was taken to be equivalent to the concentration of ET in solution.
^b Diffusion current constant of ET.

approximation of *n* (the number of electrons involved in an electrode reaction) may be obtained from calculations involving the Ilkovic equation. Such calculations, however, presuppose a knowledge of the diffusion coefficient of the substance undergoing electrochemical reaction. Since the diffusion coefficient of the reducible ET species was not known, the value of *n* could not be calculated from the Ilkovic equation. A plausible electrode reaction, derived from a consideration of the electronic nature of the reducible species is indicated by the following equation:



The variation of *E*_{1/2} values with concentration of

TABLE III—CHARACTERISTICS OF CERTAIN ET WAVES

Compound ^a ($1.00 \times 10^{-3} M$)	Description of ET Wave	$E_{1/2}$ vs SCE ^b (Volts)	Description of Residual Thiosulfate Wave
1-Dibenzylamino-2-chloropropane-HCl	Ill defined (poor plateau)		Well-defined
N-(α -Naphthylmethyl)-N-ethyl- β -chloroethylamine HCl	Well-defined	-0.97	Well-defined
N,N-Dibenzyl- β -bromoethylamine-HCl	Well-defined	-1.05	Well-defined
N-(o -Isopropylphenoxyethyl)-N-benzyl β -bromoethylamine HBr	Well-defined	-1.04 ₅	Well-defined
1-Dibenzylamino-2-bromopropane-HBr	Ill-defined		Well-defined
N,N-Dibenzyl- β -iodoethylamine-HCl	Well-defined	-1.05	Irregular

^a Compound used to form the ET derivative by reaction with thiosulfate^b $E_{1/2}$ values for ET waves obtained in solutions containing $1.0 \times 10^{-3} M$ of the amine listed and an excess of thiosulfate

the reducible species and the shape of the waves indicate that the electrode process is irreversible

DISCUSSION

These experiments show that polarography, when ever applicable, may be used to measure the relative rate of cyclization of Dibenamine-like compounds as well as the amount of ET formed by the reaction of ethylenemmonium intermediates with thiosulfate. Both of these procedures are based on the rapid thiosulfate reaction.

The relative rate of cyclization of β -haloethyl groups can be measured indirectly by polarographically following the rate at which thiosulfate is consumed by the reaction, providing this reaction is not extremely rapid. The procedure described for kinetic studies is not applicable to β -iodoethylamines since iodide ions produce polarographic waves which interfere with the thiosulfate wave. This interference is probably due to the fact that anodic iodide waves are formed at more negative potentials than chloride or bromide waves (10).

The physiologically inactive compound, dibenzylaminoethanol, did not consume thiosulfate and consequently an ET reduction wave was not obtained. Polarographic data of this kind may serve as an initial screening index of the pharmacological activity of Dibenamine-like compounds, assuming that the ethylenemmonium intermediates are responsible for the pharmacological activity of these compounds.

On the basis of a limited number of experiments no correlation could be drawn between the $E_{1/2}$ values of certain ET derivatives and their pharmacological activity as tabulated by Ullhot and Kerwin in (3).

SUMMARY

1. A polarographic method has been developed for measuring the relative rates of cycli-

zation of certain β -haloethylamine adrenergic blocking agents

2. The end product resulting from the reaction of some physiologically active Dibenamine-like compounds with thiosulfate was found to give well-defined polarographic waves upon reduction in 60% ethanol, buffered with acetate at an apparent pH of 7.0. The diffusion current obtained by reduction of the thiosulfate substitution products of Dibenamine and Dibenzylamine was found to be proportional to various concentrations of the reducible species, as well as the original concentration of the parent β -haloethylamine of the reaction mixture.

3. The physiologically inactive β -haloethylamine, dibenzylaminoethanol, did not consume thiosulfate or form a reducible thiosulfate substitution product.

4. A two electron reduction has been postulated and the ET waves appear to be irreversible.

REFERENCES

- (1) Nickerson, M., and Goodman, L. S., *Proc. Am. Fed. Clin. Research* **2**, 109 (1945).
- (2) In press.
- (3) Blicke, F. F., and Suter, C. M., "Medicinal Chemistry Vol. 2," J. Wiley & Sons, Inc., New York, 1956, p. 234.
- (4) Lord, N. G., and Christian, J. E., *THIS JOURNAL*, **45**, 530 (1958).
- (5) Lingane, J. J., and Latinen, H. A., *Ind. Eng. Chem., Anal. Ed.* **2**, 504 (1939).
- (6) Carritt, D. E., Ph.D. Thesis, Harvard University, 1947.
- (7) Meites, L., and Meites, T., *Anal. Chem.*, **20**, 984 (1948).
- (8) Sutton, I., and Grant, J., "A Systematic Handbook of Volumetric Analysis," Butterworths, London, 1955, p. 188.
- (9) Frost, A. A., and Pearson, R. G., "Kinetics and Mechanism," J. Wiley & Sons, Inc., New York, 1953, p. 47.
- (10) Kolthoff, I. M., and Miller, C. S., *J. Am. Chem. Soc.*, **63**, 1405 (1941).

The Suitability of Barley as a Host Plant for the Cultivation of Ergot*

By V. E. TYLER, Jr.†

Barley was inoculated with a specific strain of *Claviceps purpurea* using a puncture board and the resultant sclerotia were compared with those obtained from rye plants under similar conditions. As a host, rye proved superior to barley in terms of the percentage of plants infected and quantity and weight of the sclerotia produced. Likewise the alkaloid content of the rye sclerotia was slightly higher than of those obtained from barley. It is concluded that barley is inferior to rye as a host plant for the cultivation of ergot.

RYE, *Secale cereale* L., continues to be recognized as the sole official source of ergot, *Claviceps purpurea* (Fries) Tulasne (1), although the medicinally useful alkaloids may be obtained from sclerotia produced on other plants. This reflects the role of rye as the most common host of the fungus.

The proclivity of rye toward infection in comparison with other cereal grains such as wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., has been reviewed by Guggisberg (2). One of the chief considerations is the fact that under natural conditions infection by conidia is chiefly confined to the flowering period, and rye is largely dependent on cross-fertilization which requires the opening of the glumes and exposure of the gynoceium. Also, it has been established that most of the flowers of wheat and barley are already self-fertilized when the blossoms open, and chance of infection with ergot spores is thereby greatly reduced.

The characteristics of ergot existing as a natural parasite on various members of the *Gramineae* have been the subject of a number of investigations (3-5). Recently, Meinicke (6) has reported on the alkaloid content of ergot produced by the inoculation of various wild grasses with a specific strain of *Claviceps purpurea*. The results of his experiments indicate that the host may influence the alkaloid content of the parasite in a quantitative manner only. It is apparent, therefore, that quantitative reports of the alkaloid content of ergot sclerotia formed on different grasses are not comparable, one with another, unless it is known that both hosts were infected with the identical strain of the fungus.

With the advent of infection methods which

are not dependent upon the periodic opening of the blossoms of the various plants (7) and the availability of specific ergot strains obtained from single-spore cultures, it appeared of interest to compare the quantity and quality of ergot produced by puncture-inoculation of rye and another host plant under experimental conditions.

The availability of a variety of barley, producing plants about one-third of which were male sterile and therefore quite susceptible to infection on theoretical grounds, accounted for its selection as the plant to be compared with rye under field conditions with respect to its relative suitability as a host for ergot.

EXPERIMENTAL

Inoculation.—Winter rye, *Secale cereale* var. *caribou* and spring barley, *Hordeum vulgare*, heterozygous for male sterility, were planted in rows 10 feet long, spaced 12 inches apart. The rows selected for inoculation were part of a series of larger plots employed for general agronomic research and received no special treatment. Although widely separated, the two plots were quite similar in terms of soil, drainage, exposure, and related factors. In order to obtain samples large enough to be significant, plots containing approximately 1,000 spikes of each grain were treated.

The inoculum was prepared by inoculating 1 inch \times 8 inch culture tubes, containing slants of a casein hydrolysate-mannitol-basis nutrient medium (8) solidified with 2% agar, with an ergotamine-producing strain¹ of *C. purpurea* originally selected from a mono-conidiospore isolate. These cultures were prepared four to six weeks prior to need and stored at 25°, during which time a profuse mycelial development took place. The mycelial mat was then removed from 4-6 slants, depending upon the quantity of growth present, homogenized in a Waring Blendor with a small quantity of distilled water and this slurry diluted to a volume of 480 ml. This constituted the finished inoculum which was applied to the plants with a puncture board as described by Guggisberg (9). The puncture board employed in this investigation measured approximately 12 \times 13 cm. and was set with eyed needles which projected

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¹ Supplied through the courtesy of Dr. N. Bekesy, Budapest, Hungary

5-7 mm. from its surface. The needles were evenly spaced and averaged seven per sq. cm. of board surface.

By May 24, 1957, the winter rye had matured sufficiently so that most of the plants were in the developmental stages labeled I, II, and III by Bekesy (10). Inoculation was carried out late in the afternoon so that the spores might receive the benefit of the cool night hours during their initial germination stages. The spring barley was considered ready, and consequently inoculated, on June 10, the majority of plants having reached stages I and II by this date. The fact that barley is known to undergo self-fertilization before emergence of the spike from the sheath accounted for the inoculation of this plant at a somewhat earlier stage of development.

No unusual weather conditions were noted subsequent to the inoculations and the fungus appeared to develop normally on both hosts, honeydew being evident after six or seven days. It has been established by Loo and Lewis (11) that ergot sclerotia reach a maximum alkaloid content after nineteen days of development although they are still increasing in weight at this time. Therefore, in this experiment a growing period of one month was arbitrarily selected as being sufficient time to allow most sclerotia to mature, at least to maximum alkaloid content, but not long enough to allow appreciable losses to occur by quantities of large ripe sclerotia falling from the drying spikes of the plants. At this time all of the spikes in each of the experimental plots were harvested, regardless of infection, and subjected to analysis.

Analytical Procedures.—Total spikes were counted as were the number infected with the fungus. The sclerotia were then separated, the maximum number in a single spike being noted during the process. All sclerotia were counted, the entire sample weighed and its moisture content determined by a conductometric method (12). Samples of each type of ergot were then ground to a No. 20 powder in a Wiley Laboratory Mill and fat determinations carried out on duplicate 2-Gm. samples as described by Jones and Tyler (13). The defatted samples were next milled to a No. 60 powder and duplicate samples assayed for water-soluble and water-insoluble alkaloids by the procedure of Silber and Schulze (14). Water-soluble alkaloids were calculated as ergonovine and reported on the basis of a moisture-free, fat-free sample. The water-insoluble alkaloids were calculated as ergotoxine and reported on the same basis. Portions of the water-insoluble extracts not used for assay were made alkaline, extracted with ether and these ethereal extracts chromatographed by the method of Mciniche (6) to determine the composition of the water-insoluble alkaloid fraction. The water-soluble fractions treated in the same manner were chromatographed in a butanol-acetic acid-water (4:1:5) system as described by Tyler and Schwarting (15) to determine their composition. The results of these analyses are recorded in Table I.

DISCUSSION

These data indicate that rye is superior to barley as an ergot host plant in terms of quantity and weight

TABLE I.—COMPARISON OF THE ERGOTS PRODUCED ON RYE AND BARLEY

	Rye	Barley
Total no. of spikes	1,115	989
No. of infected spikes	722	211
Infected spikes (%)	64.8	21.3
No. of sclerotia	2,371	1,159
Av. no. sclerotia per infected spike	3.3	5.5
Max. no. sclerotia per infected spike	22	28
Fresh wt. sclerotia (Gm.)	57.27	22.44
Av. wt. of single sclerotium (mg.)	24.1	19.4
Av. wt. of ergot per infected spike (mg.)	79.3	106.4
Moisture (%)	6.7	7.4
Fat (%)	15.6	10.23
Alkaloid—water-sol. (%)	0.045	0.027
Alkaloid—water-insol. (%)	0.631	0.515
Alkaloid identity—water sol.	Ergonovine Ergotamine (90% of complex) Ergosine (little) Ergotamine (little) Ergocristine — Ergocornine (faint trace) Ergokryptine (trace)	
Alkaloid identity—water-insol.		

of sclerotia formed in the total plots. Nearly two-thirds of the rye spikes treated developed one or more sclerotia in comparison to slightly more than one-fifth of the barley spikes, and the yield from the entire rye plot was more than double that of the barley on a weight basis. The individual rye sclerotia were slightly larger and contained a slightly higher alkaloid content than those of barley although the qualitative composition of the alkaloidal mixture of both ergots was identical. On the other hand, the barley plants which did become infected produced more sclerotia (av. 5.5) per spike than the rye (av. 3.3). This seems to indicate that the difference in these two plants as hosts for ergot is due to the greater susceptibility of the rye to infection, even when both are infected by an injection technique which circumvents the effects produced by differences in flowering habits. Apparently this greater susceptibility applies to the rye plants as a group but not necessarily to the individual since a single, infected barley plant yielded, on the average, more ergot sclerotia both in number and total weight than a single infected rye plant; however, fewer barley plants in the group became infected.

The reasons for these differences in susceptibility of the two plants are obscure although the sterile character of the variety of barley employed was probably not without influence, particularly on the number of sclerotia which developed on the individual barley spikes. Garay (16) has investigated some of the factors which affect the germination of ergot conidia with reference to the host plant. Hydrogen ion concentration and osmotic pressure were shown to have marked effects on germination, but no stimulation could be shown to result following treatment with extracts of rye tissues. The same extracts, after autoclaving, did stimulate growth of saprophytic cultures of ergot, thus confirming the report of Berman and Youngken (17). The impor-

tance of these various factors with reference to this experiment is merely speculative, but they may be used as a starting point for further study on the suitability of various cereals as hosts for the cultivation of ergot

REFERENCES

- (1) "The National Formulary 10th ed J B Lippincott Co Philadelphia 1954 p 216
- (2) Guggisberg H Mutterkorn Vom Gift zum Heilstoff S Karger Basel 1954 pp 14-17
- (3) Rochelmeyer H *Pharmazie* 4, 326(1949)
- (4) Boros A and Garay, A, *Botanikai Közlemények* 46, 83(1955)

- (5) Silber, A, and Bischoff, W, *Arch Pharm*, 288, 121 (1955)
- (6) Meimcke, R *Flora (Jena)* 143, 395(1956)
- (7) Bekesy, N, *Zentr Bakteriell Parasitenk Abt II*, 99, 321(1938)
- (8) Tyler, V E, Jr, and Schwarting, A E, *THIS JOURNAL* 41, 590(1952)
- (9) Guggisberg H, *op cit* pp 27-28
- (10) Bekesy, N *Pharmazie* 11, 339(1956)
- (11) Loo, Y H, and Lewis R W, *Science*, 121, 367(1955)
- (12) Wier, J K, and Tyler, V E, Jr, *Drug Standards* 24, 117(1956)
- (13) Jones, D D, and Tyler, V E, Jr, *THIS JOURNAL* 44, 480(1955)
- (14) Silber, A, and Schulze T, *Pharmazie* 8, 675(1953)
- (15) Tyler, V E, Jr, and Schwarting A E *THIS JOURNAL* 41, 354(1952)
- (16) Garry, A *Physiol Plantarum* 9, 350(1956)
- (17) Berman M, and Youngken, H W Jr, *THIS JOURNAL*, 43, 200(1954)

A Quantitative Papergram Assay Method for Prednisolone in Tablet Formulations*

By P. D. MEISTER, C. A. SCHLAGEL, J. E. STAFFORD, and J. L. JOHNSON

The quantitative assay of prednisolone in tablet formulations herein described involves separation of the steroid from the nonsteroidal tablet components by paper chromatography. Elution of the steroid spot from the papergram with methanol is followed by measurement of the ultraviolet absorption of the eluate at 224 m μ , 244 m μ , and 260 m μ . The absorbance values measured at these three wavelengths are used to calculate the true steroid concentration. The computation provides correction for the small but significant amounts of ultraviolet absorbing materials which are invariably present in the papergram eluates

THE NEED for an assay method specific for prednisolone became apparent when tablet formulations containing prednisolone, acetyl salicylic acid, and an antacid were subjected to an accelerated stability study at 70°. In this study, assays by the conventional triphenyltetrazolium assay showed that, after an initial decrease, the prednisolone concentration appeared to increase upon prolonged exposure to 70° (1). This apparent increase indicated the formation of a reducing substance in the dosage form during the course of the heat stress.

The principle of the procedure described in this paper evolved from the methodology previously developed in our laboratories for the quantitative assay of progesterone in the presence of 11 α -hydroxyprogesterone (2). The present method involves the paper chromatographic separation of prednisolone from major undesirable components followed by spectrophotometric measurements at three different wavelengths to compensate for minor amounts of nonsteroidal absorbing substances in the eluates.

EXPERIMENTAL

Reagents and Material

Reagents—Chloroform, A R (Mallinckrodt Chemical Works), absolute ethyl alcohol, (Commercial Solvents Corporation), absolute methyl alcohol, A R (Mallinckrodt Chemical Works), propylene glycol, U S P (Carbide and Carbon Chemicals Corp.), ethylene dichloride, 99%, (Dow Chemical Company), toluene, A R (Mallinckrodt Chemical Works), acetic acid, C P (E I DuPont de Nemours & Co.), prednisolone Delta-Cortef® (Upjohn brand of prednisolone) standard solution 1 mg/cc in 95% ethanol¹, and chromatographic paper, Eaton Dickman 613, 6 inches \times 23 inches.

Apparatus.—Semimicro evaporator constructed in these laboratories, spectrophotometers—a Beckman Model DU and a Cary Recording spectrophotometer Model 14 were employed, pipet—a 0.2 cc pipet graduated in 0.01 cc (MISCO), and papergram scanner (3).

Development of the Papergram Assay Procedure

Previous experience has shown that the development of a quantitative paper chromatographic assay consists of the following steps:

Selection of a suitable solvent system—The solvent system of choice, besides giving as complete as

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¹ Dr. T. Chulski of these laboratories showed this material to be 99.82% pure by solubility analysis.

possible separation of the steroid from nonsteroidal components, should not excessively contribute to the ultraviolet absorption of the papergram eluates.

Determination of nonspecific ultraviolet absorption.—Small contributions to the ultraviolet absorption of the papergram eluates were expected to result from the elution of the paper and the solvents (2) as well as from the elution of minor tablet ingredients. Therefore, an accurate determination of the background absorption was necessary to provide for its mathematical compensation.

Determination of steroid recovery.—The average recovery of known amounts of steroid has to be determined for each solvent system.

Calculation of the true steroid concentration.—A mathematical formula has to be developed which compensates for the impurities found to be present in the papergram eluates.

Selection of a Papergram Solvent System.—A tablet formulation containing prednisolone, acetylsalicylic acid, calcium carbonate, sucrose, lactose, starch, ethylcellulose, talc, and mineral oil was exposed to 70° temperature for seventy-four hours. The neutral chloroform extract of the crushed tablets was essentially free of prednisolone as indicated by the ultraviolet and infrared spectra of the extract. This extract, however, had considerable absorbance when assayed with triphenyltetrazolium (4).

Of all the solvent systems investigated the propylene glycol-toluene-methanol system of Zaffaroni as modified by Reineke (2) with acetic acid (PTFA-system) gave the best separation of mixtures of prednisolone (32 γ), of acetylsalicylic acid (50 γ) and of the above described extract (50–100 γ). The mobile phase consists of 2.0% glacial acetic acid added to toluene previously saturated with propylene glycol. The stationary phase is propylene glycol-methanol in a 1:1 ratio.

Quantitative Determination of Nonsteroidal Background Absorption Resulting from the Elution of Papergram Blanks.—Two empty sheets properly pretreated with the stationary phase were developed with the mobile phase for thirty-nine hours. After a drying period of two hours at 108°, two parallel strips 6 cm. wide (beginning 5 cm. below the origin and extending 25 cm. downwards) were cut out. These two strips were subdivided into smaller pieces of approximately 6 × 3.5 cm. size. These latter pieces were then cut into bits of 1 cm.² size and were eluted in individual 250-cc. Erlenmeyer flasks with 50 cc. of dry methanol. The ultraviolet absorption of the eluates was determined with the Cary spectrophotometer. The results are given in Table I.

Quantitative Determination of the Background Absorption Resulting from Nonsteroidal Formulation Components.—Forty hours of development on the PTFA system gave excellent separation of prednisolone and acetylsalicylic acid. To determine the degree of interference from minor amounts of U. V. absorbing compounds six tablet formulations were prepared which contained all the ingredients except prednisolone, but which differed from each other with respect to the carbohydrate component. After storage at 70° these tablets were extracted with chloroform.

Approximately equal aliquots of the extraction residues in chloroform solution were applied to paperstrips and the strips were developed and processed

TABLE I.—BACKGROUND ABSORPTION RESULTING FROM THE ELUTION OF BLANK PAPERGRAMS

Sample	A 224 mμ	A 244 mμ	A 260 mμ
A1	0.042	0.015	0.019
A3	0.020	0.002	0.004
A5	0.010	0.002	0.006
A7	0.048	0.015	0.020
B1	0.012	0.002	0.005
B2	0.018	0.003	0.007
B4	0.020	0.004	0.007
B6	0.040	0.012	0.014
B8	0.046	0.020	0.020
Average Ratios: A 224 mμ/A 244 mμ = 3.41; A 260 mμ/A 244 mμ = 1.36			

as described in the final assay method. Areas (approximately 6 × 3.5 cm.) were cut from the dried sheets which corresponded in size and position to the size and position of the areas occupied by the prednisolone spots in the assay procedure. Each area was then cut into small bits of 1 cm.² size and eluted with 30 cc. of methanol.

The ultraviolet absorption of the eluates was determined with the Cary spectrophotometer. Visual inspection of the curves indicated that all of them had a similar shape with one of the maxima being at 260 mμ. The absorbances at 224 mμ, 244 mμ, and 260 mμ are given in Table II.

TABLE II.—BACKGROUND ABSORPTION RESULTING FROM PAPERGRAM ELUTION OF NONSTEROIDAL TABLET COMPONENTS

Carbo- hydrate	Time Ex- posure to 70°, Hr.	Aliquot of Ex- tract Applied to Paper, mg.	Absorbance of Papergram Eluates ^a		
			A 224 mμ	A 244 mμ	A 260 mμ
Starch	52	0.982	0.090	0.042	0.039
Lactose	52	0.970	0.029	0.022	0.014
Methyl- cellulose	52	0.994	0.020	0.014	0.012
Sucrose	52	1.015	0.013	0.008	0.007
D-Sorbitol	52	0.959	0.030	0.020	0.013
Dextrose	52	0.975	0.005	0.004	0.002
Dextrose	76	1.003	0.023	0.016	0.015
Average Ratios: A 224 mμ/A 260 mμ = 2.04; A 244 mμ/A 260 mμ = 1.17.					

^a These values have been corrected for the average blank absorption (cf. Table I) at each wavelength.

Determination of Steroid Recovery.—Two aliquots of a methanolic solution containing 4.00 mg. of prednisolone were applied to the paper in the manner described under the final assay method. After forty hours development in the PTFA-system, each prednisolone spot was eluted from the paper with 30 ml. of methanol. The prednisolone concentrations were determined at 244 mμ and the values corrected for the average background absorption eluted from the paper (cf. Table I). Two sets of experiments each consisting of six determinations gave an average recovery of 95.1% which is in good agreement with the previously reported recovery of progesterone (2).

Calculation of Prednisolone Concentration in Papergram Eluates.—*Formula I.*—While the papergram eluates contained three ultraviolet absorbing components (x, y, and z, below), only the concen-

tration of the steroid was of interest. This required absorbance readings at three wavelengths and the use of a general three component equation for the desired steroid component, λ , in the three component system according to known procedures (5). The equation for the steroid concentration was thus

$$C_x = \frac{A_1(a_{2y}a_{3z} - a_{3y}a_{2z}) + A_2(a_{3y}a_{1z} - a_{1y}a_{3z}) + A_3(a_{1y}a_{2z} - a_{2y}a_{1z})}{a_{1z}a_{2y}a_{3z} - a_{1z}a_{3y}a_{2z} - a_{2z}a_{1y}a_{3z} + a_{2z}a_{3y}a_{1z} + a_{3z}a_{1y}a_{2z} - a_{3z}a_{2y}a_{1z}}$$

Where λ = absorption due to prednisolone, y = absorption of the paper blanks, z = absorption contributed by nonsteroid tablet components, a = absorptivity, 1 = absorbance, C = concentration in Gm/liter, and 1, 2, 3, = 224, 244, 260 $m\mu$, respectively.

Since in the present problem the absolute quantities of the components y and z are of no interest the ratio values derived from Tables I and II are substituted for the corresponding a values. The reference data for prednisolone in methanol are

$$a_{224} m\mu = 24.55, a_{244} m\mu = 41.12, a_{260} m\mu = 28.79$$

With these values the following equation was obtained

$$C_x = \frac{1.95A_3 - 0.64A_1 - 0.59A_2}{15.48}$$

where C_x = concentration of prednisolone in Gm/L.

Incorporation into the equation of the 95.1% recovery of the steroid from the papergram gave the final form of the equation

$$C_{\text{prednisolone}} = \frac{1.95A_3 - 0.64A_1 - 0.59A_2}{14.72} \quad (\text{Eq. 1})$$

This formula was applied to the determination of prednisolone in stability samples of a large number of tablet formulations. The results showed conclusively that in many instances the triphenyltetrazolium assay gave higher potencies of prednisolone than the papergram assay. The results of the papergram assay, therefore, were used to screen for the more stable formulations.

Formula II—This screening process showed that a double compressed tablet possessed excellent stability properties. It was considered necessary to determine the background absorption of this specific formulation to increase the accuracy of the method for the assay of the stability samples. Steroid free tablets were, therefore, made and subjected to 70° for 24, 48, and 72 hours. Extractions of the tablet cores, paperchromatography of the extracts, and determination of the ultraviolet absorption of the papergram eluates gave the values listed in Table III.

When these ratios and the 95.1% recovery value were incorporated into the above mentioned general equation the following formula was obtained

$$C_{\text{prednisolone}} = \frac{0.07A_1 + 1.05A_2 - 0.44A_3}{21.36} \quad (\text{Eq. 2})$$

This formula was used for the results discussed and analyzed in the subsequent paper (6).

Final Assay Method

Tablet Extraction.—Ten tablets were taken as the sample for analysis to reduce sampling error. The cores containing the steroid were separated

from the outer coat, weighed, and finely powdered. Three aliquots were weighed with each aliquot corresponding to three tablets. One of these aliquots was used for the papergram assay and two for the two tetrazolium assay procedures "B" and "C" (6).

The aliquot was slurried in 10 cc of deionized

water and 2.5 cc of a 25% sodium carbonate solution. The slurry was extracted with 50, 30, and 30 ml of chloroform, respectively. The extracts were evaporated in the semimicro evaporator. The residue was transferred into 1 cc volumetric flasks with small amounts (approximately 1 cc) of a 1:1 mixture of chloroform:ethylene dichloride. Each transfer volume was evaporated by passing a stream of purified nitrogen through the volumetric flask. The final volume was adjusted to 1 cc.

Paper chromatography.—Duplicate spots each made from 0.1 cc of the same sample were applied to the "origin" of one paper sheet. Each spot occupied a site 4 cm in length which was so spread that 2-cm and 3-cm blank spaces remained between each spot and the long edge of the paper, and between the two spots, respectively. The descending papergram was developed in an air tight battery jar. After approximately forty hours of development, the sheets were removed from the development chamber, dried in a hot air oven for two hours at 105°, and processed as described in detail by Remeke (2).

An aliquot of the eluate was read in the Beckman DU spectrophotometer.

The absorbance values which were obtained from the duplicate analysis of an authentic sample are given in Table IV.

TABLE III—NONSTEROIDAL BACKGROUND ABSORPTION RESULTING FROM PAPERGRAM ELUTION OF A SPECIFIC TABLET FORMULATION

Sample	Time Exposed at 70°	A 224 $m\mu$	A 244 $m\mu$	A 260 $m\mu$
1A	24	0.034	0.011	0.010
1B	24	0.044	0.017	0.017
2A	48	0.069	0.032	0.029
2B	48	0.045	0.018	0.017
3A	74	0.055	0.022	0.020
3B	74	0.035	0.017	0.017
Average Ratios A 224 $m\mu$ /A 260 $m\mu$ = 2.56, A 244 $m\mu$ /A 260 $m\mu$ = 1.06				

These values were then inserted into equation 2, thus

$$C_{\text{prednisolone}} = \frac{0.07 \times 0.642 + 1.05 \times 0.452 - 0.41 \times 0.408}{21.36} = \frac{0.3400}{21.36} = 1.59 \times 10^{-2} \text{ Gm/L}$$

The concentration of prednisolone for one tablet was calculated from the above data in the following manner. Since an aliquot a (0.1) of the extractives from b (3) tablets was applied to the papergram and

TABLE IV.—ABSORBANCE VALUES FROM PAPER-GRAM ELUATES OF AN AUTHENTIC SAMPLE

Sample Aliquot	A ₁ (224 mμ)	A ₂ (244 mμ)	A ₃ (260 mμ)
2A	0.408	0.612	0.452
2B	0.398	0.623	0.442

the steroid spot was eluted with *d* (0.03) liters of solvent, the calculation became:

$$C_{\text{prednisolone/tablet}} = \frac{C_{\text{prednisolone}} \times d}{a \times b} \text{ Gm.} = \frac{1.59 \times 10^{-3} \text{ Gm./L.} \times 3 \times 10^{-2} \text{ L.}}{10^{-1} \times 3} \text{ or } C_{\text{prednisolone/tablet}} = 1.59 \times 10^{-3} \text{ Gm}$$

Table V gives a comparison of values which were obtained by different procedures of calculation from the absorbances listed in Table IV

TABLE V.—CALCULATION OF PREDNISOLONE CONCENTRATION (MG/TABLET) FROM ABSORBANCES OF TABLE IV BY DIFFERENT PROCEDURES

Sample Aliquot	Method of Calculation		
	A ^a	B ^b	C ^c
2A	1.59	1.56	1.47
2B	1.56	1.51	1.42

^a Calculated according to Formula II

^b Calculated from A₂ (244 mμ) alone without consideration of any correction for background absorption or of 95.1% recovery from the paper

^c Calculated from A₂ (244 mμ) alone after correction of A₁ for background absorption eluted from the paper and with consideration of 95.1% recovery of steroid from papergram

Table V shows that calculation of the steroid concentration by formula II gives the highest values. That these values are very close to the true concentration was determined by processing two accurately weighed samples of prednisolone through the whole analytical procedure. The final results were 2% and 3% low, respectively.

DISCUSSION

A reproducible paperchromatographic assay has been developed which is highly specific for prednisolone in tablet formulations. Although the assay is rather tedious it is superior to conventional methods of 17-hydroxycorticosteroid assay because it almost completely eliminates tablet components and degradation products which might interfere with other methods. The method is designed in such a way that it compensates for minor interferences which are still present after prolonged paper chromatographic separation. Therefore, it is ideal for establishing the veracity of other, less tedious methods.

The paper chromatographic method can also be used for the qualitative determination and characterization of possible artifact material. This may be helpful in uncovering and interpreting incompatibilities between components in the formulation. To do this, the development of the papergram is interrupted before the artifact spot disappears from the end of the sheet. For example, an inferior prednisolone-acetylsalicylic acid formulation was subjected to the paper chromatographic assay procedure. An artifact area was eluted from the papergram which was subsequently identified by micro-infrared spectroscopy as prednisolone acetate (7). This indicated an undesirable transesterification between prednisolone and acetylsalicylic acid. Knowing the nature of these possible interactions facilitates the formulation of a stable product.

This paper chromatographic assay should be applicable, with minor modifications, to the determination of prednisolone in many other dosage forms.

SUMMARY

1. A quantitative paper chromatographic assay for prednisolone in tablet formulations has been developed and applied to the development of a stable prednisolone formulation containing acetylsalicylic acid.

2. Because of its specificity this assay is superior to the triphenyltetrazolium assay in the presence of tablet ingredients which interfere with the latter method.

3. The assay is not recommended for the routine analysis of tablet formulations with good stability. Its application is advantageous, however, if the reliability of routine assay such as the triphenyltetrazolium method has to be established.

4. The versatility of the paper chromatographic assay is discussed in relation to the problem of qualitative interpretation of incompatibilities.

REFERENCES

- (1) Garrett, E. R., and Booth, R. E., These Laboratories, private communication.
- (2) Reineke, L. M., *Anal. Chem.*, **28**, 1853(1956).
- (3) Drake, N. A., Haines, W. J., Knauff, R. E., and Nielson, E. D., *ibid.*, **28**, 2036(1956).
- (4) Wagner, J. G., These Laboratories, private communication.
- (5) Friedel, R. A., and Orchin, M., "Ultraviolet Spectra of Aromatic Compounds," John Wiley & Sons, Inc., New York, 1951, p. 29.
- (6) Wagner, J. G., Dale, J. K., Schlagel, C. A., Meister, P. D., and Booth, R. E., *This Journal*, **47**, 580(1958).
- (7) Shell, J., These Laboratories, private communication.

Comparison of Methods of Assay of Prednisolone in Compressed Tablets*

By J. G. WAGNER, J. K. DALE, C. A. SCHLAGEL, P. D. MEISTER, and R. E. BOOTH

A paper chromatographic method and a method based on chloroform extraction followed by a tetrazolium assay are shown to give similar results in the determination of prednisolone in certain compressed tablets after storage at room temperature and at elevated temperatures. A method based on alcoholic extraction of the steroid followed by a tetrazolium assay is shown to give higher values of potency than the other two methods on most of the stability samples. The increased stability of prednisolone in compressed tablets when the tablets are stored with a desiccant is noted.

THIS COMMUNICATION is concerned with a comparison of three methods of assay of prednisolone in compressed tablets. Method "A" is the paper chromatographic procedure of Meister, *et al.* (1), which without modification can be used for the assay of tablets containing both reducing sugars and acetylsalicylic acid. Method "B" is a chloroform extraction-tetrazolium method which can be used to assay tablets containing reducing sugars and can be easily modified to assay tablets also containing acetylsalicylic acid. Method "C" is an alcohol extraction-tetrazolium method which is unsatisfactory when a reducing sugar such as lactose is present or when applied to certain aged prednisolone tablets.

Previously described colorimetric methods for the determination of 17-hydroxycorticosteroids involve the reduction of a tetrazolium salt in alkaline solution by the ketol group of these steroids (2-8).

Mader and Buck (2) applied the tetrazolium color assay to various steroids and reported that cortisone acetate could be separated from reducing sugars such as lactose by extraction of the steroid with absolute ethyl alcohol or isopropyl alcohol. We have found that the solubility of lactose in absolute ethyl alcohol is sufficient to cause considerable interference in the tetrazolium assay of prednisolone.

The chemical reduction of 2,3,5-triphenyltetrazolium chloride (tetrazolium salt) in alcoholic solution by prednisolone in the presence of approximately 0.009 *N* tetramethylammonium hydroxide is rapid and essentially complete after thirty to thirty-five minutes. Providing only prednisolone is present the absorbance of the colored solutions at the wavelength of maximum absorption (485 m μ) remains essentially constant over the range of thirty-five to sixty minutes. An increase in absorbance with time,

in the range of thirty-five to sixty minutes, indicated that an interfering nonsteroidal substance is present. Izzo, Burton, and Keutmann (7) reported similar data for other steroids possessing an alpha ketol side chain. The values of prednisolone potency reported below were calculated from the plateau values of absorbance, i. e., from the absorbance values of the sample and standard after the level portion of the absorbance *versus* time curve had been reached.

EXPERIMENTAL

Reagents.—*Tetrazolium Solution.*—A 0.5% w/v solution of 2,3,5-triphenyltetrazolium chloride (Dajac Laboratories) in absolute alcohol.

Base Solution.—One milliliter of a 10% aqueous solution of tetramethylammonium hydroxide (Eastman Organic Chemicals No. 1515) was diluted with 9 ml. of absolute alcohol. The solution was filtered through Whatman No. 1 filter paper.

Standard Prednisolone Solution.—A solution containing 10 μ g. of specially purified prednisolone per ml. of absolute alcohol. Prednisolone, absolute ethyl alcohol, and chloroform were the same as described by Meister, *et al.* (1).

Samples.—Prednisolone tablets, containing no lactose nor acetylsalicylic acid, were prepared from the same formula but by two different processes. Those prepared by one process are designated Lots I and IA and those prepared by the other process are designated Lots II and IIA. The results reported in Table I led to the following sampling technique. Three aliquots, each representing three 1.5-mg. tablets, were taken from 10 finely powdered tablets. One of the aliquots was assayed by method "A," one by method "B," and one by method "C." Assay values plotted in Figs. 1 through 4 and tabulated in Table II resulted from this type of sampling.

Assay Methods.—*Method "A"* was the paper chromatographic method reported by Meister, *et al.* (1).

Method "B."—The aliquot of powder was mixed with 4 ml. of deionized water in a 500-ml. volumetric flask. About thirty minutes later approximately 250 ml. of chloroform was added, the flask was stoppered, shaken for about two minutes, then allowed to stand for fifteen to thirty minutes. Sufficient chloroform was added to bring the volume to the bottom of the neck, the flask was stoppered and

* Received November 20, 1957, from The Upjohn Company, Kalamazoo, Mich.

shaken again for about two minutes. Chloroform was added to the fiducial mark of the flask. The flask was stoppered, shaken, and allowed to stand until the layers separated. After removing the water layer above the fiducial mark with a vacuum hose two 20 ml aliquots were withdrawn from the center of the flask. Each aliquot was transferred to a 25 ml glass stoppered flask containing three glass beads. Most of the chloroform was evaporated on a steam bath but the last traces were removed by suction. The steroid residue was dissolved in 20 ml of absolute ethyl alcohol, 20 ml of tetrazolium solution were added followed by 20 ml of base solution. The contents of the flask were mixed by swirling. After thirty five and forty-five minutes the absorbance was determined at 485 m μ on a Beckman spectrophotometer, Model DU. A standard solution of prednisolone and a blank consisting of absolute alcohol were carried through the identical procedure.

Method "C"—The aliquot of powder was transferred to a 500-ml volumetric flask with the aid of 250 ml of absolute alcohol. The flask was stoppered and shaken in a shaking machine for two and one half hours which was shown to be sufficient for complete extraction of the steroid. The flask was then made up to volume with absolute alcohol and the contents mixed by inversion. An aliquot of about 30 ml of the extract was centrifuged at 20,000 r p m until the supernatant was optically clear. The remainder of the procedure was identical to that described for method "B" except that one-half the volumes of alcoholic solution and reagents and a Beckman spectrophotometer, Model B were used.

For methods "B" and "C" potency of the tablets was calculated by means of the equation

$$\text{Milligrams of prednisolone per tablet} = \frac{5}{3} \times (A_{\text{sample}}/A_{\text{standard}})$$

where A_{sample} is the absorbance of the sample at 485 m μ in the plateau region and A_{standard} is the absorbance of the standard at the same wavelength and after the same time.

RESULTS AND DISCUSSION

Freshly Made Tablets—The three methods of assay gave the same average potency of prednisolone in freshly made tablets. Table I shows the results obtained with Lot I by methods "B" and "C" and with Lot IA by all three methods. The average of the two series of assays obtained by method "C" on Lot I were tested against the average obtained by method "B" on Lot I by Student's *t* test (9a). Similarly the averages of the two series of assays obtained by method "C" on Lot IA were tested against the averages obtained by method "B" on Lot IA. In both cases the *t* values were less than unity and no significant difference between averages by the two methods could be shown. Method "A" gave identical results in the case of Lot IA, the range being 1.53 ± 0.01 whereas the 95% confidence interval about the mean for method "B" was 1.52 ± 0.03 and for method "C" was 1.53 ± 0.02 .

The repetitive assays on groups of three tablets of Lots I and IA by methods "B" and "C" disclosed that the distribution of prednisolone per tablet in

Lot IA was much more uniform than in Lot I. This was confirmed by comparing the standard deviations associated with samples (10) for Lots I and IA. To reduce this sampling error in subsequent assays the sampling procedure discussed under "Samples" was used in all subsequent investigations. Duplicate assays were run on each extract by each method in order to estimate precision.

Short Term Stability Tests Carried Out at 70°—In order to quickly screen formulas, methods of preparation, and packaging variations, samples of the tablets were stored at 70° in an air-circulating oven equipped with an automatic temperature recorder for varying periods up to seventy two hours.

A blank tablet prepared with the same formula and method of preparation as Lots I and IA but without prednisolone was stored at 70° in sealed bottles with metal-liner caps without Humi Caps¹ for twenty four, forty-eight, and seventy-two hours. The samples were analyzed by methods "A" and "C". Results of analysis of these samples by method "A" were incorporated in the three component equation used to calculate potency on the other samples (1). By method "C" the twenty-four and forty eight hour samples of these tablets gave a "potency" equivalent to 0.03 mg of "prednisolone" per tablet and the seventy-two hour sample gave a "potency" equivalent to 0.08 mg of "prednisolone" per tablet.

Tablet lot IA, stored at 70° with Humi Caps in the bottles, when assayed by method "A" and "B" exhibited a loss of 9.2% of the initial prednisolone content (1.53 mg/tablet) during the first twenty four hours but no more loss in potency during the next forty-eight hours. Results obtained with method "C" on the same samples were all much higher, the mean value being 1.56 mg of prednisolone per tablet in the twenty four to seventy-two hour range. These results are plotted in Fig 1.

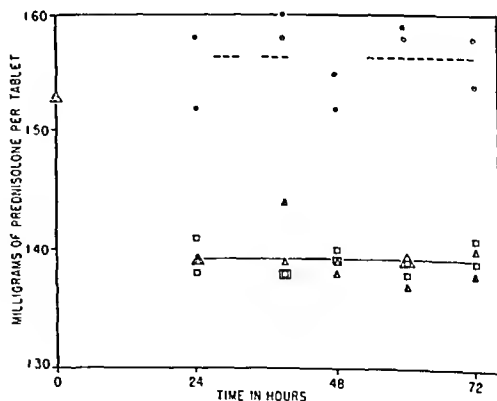


Fig 1—Plot of milligrams of prednisolone per tablet against time in hours for lot IA stored at 70° in sealed bottles with metal-liner caps and Humi Caps. Δ —Method "A", \square —method "B", \circ —method "C".

¹ Humi Caps—A plastic container enclosing approximately 0.75 Gm. of dried silica gel manufactured by Dri Aire Incorporated, South Norwalk, Conn.

TABLE I—COMPARISON OF ASSAY RESULTS OBTAINED BY THREE DIFFERENT METHODS AND INVESTIGATORS ON DIFFERENT SAMPLES OF THE SAME FRESHLY MADE TABLETS

		Potency Milligrams of Prednisolone Per Tablet					
Method B ¹	Lot I ^a	Method C	Method B	Lot IA ^a	Method C	Lot IA ^b	Method A ¹
1 48	1 61	1 57	1 48	1 51	1 50	1 52	1 54
1 43	1 43	1 40	1 52	1 47	1 47	1 53	
1 51	1 54	1 50	1 53	1 53	1 53	1 54	1 53
						(Av 1 53)	
1 48	1 55	1 56	1 53	1 55	1 52		
1 60	1 59	1 60	1 55	1 57	1 56		
1 53	1 51	1 51	1 49	1 56	1 53		
(Av 1 51)	(Av 1 53)		(Av 1 52)	(Av 1 53)			

^a In each case six groups of three tablets were extracted by each method. Only one tetrazolium assay was done on each extract by method B but two tetrazolium assays were done on each extract by method C.

^b Ten tablets were ground to a powder and three aliquots of powder each representing three tablets were extracted separately. Two paper strips were run on each extract. One paper strip was damaged and could not be used.

Results obtained when tablet lot IA was stored at 70° without Humi Caps in the bottles are plotted in Fig 2. The values obtained by method "C" were corrected for the values of "prednisolone" found for the blank tablets. It can be seen that prednisolone in lot IA, stored at 70° in the absence of Humi Caps, shows a linear degradation with time by all these methods. Linearity of such a plot indicates a constant degradation rate independent of concentration, i e., pseudo zero order rate. The slopes of the lines shown in Fig 2 were calculated by the method of "least squares" (9b) and the slopes were compared by calculating the value of $t = \text{difference in slopes} / \text{standard deviation of the difference}$ (9c). The slopes of the lines do not differ at $P = 0.05$. However, there is obviously a bias in the results obtained by method "C".

The improvement in stability of prednisolone in

tablets when the tablets are stored with a desiccant (Humi Caps or Dri Pax²) is noteworthy. In the 70° study with Humi Caps the loss in potency which occurs during the first twenty four hours may be ultimately caused by moisture in the tablet. The water is finally absorbed by the silica gel, and, once equilibrium is attained, no further loss in potency occurs. At 47° the improvement in stability when a desiccant is present is also evident.

One and Two Month Stability Samples—Table II is a tabulation of averages of duplicate assay values obtained by the three methods on aliquots of the same two month stability samples of Lots I and II and aliquots of the same one month stability samples of Lots IA and IIA. Tablets of the four lots were stored in sealed bottles under the conditions indicated in the table.

Evaluation of Data—The average deviation of duplicate assays on 23 samples was 0.02 mg of prednisolone for all methods, the 95% limits were 0.05 mg of prednisolone per 1.5 mg tablet which is an error of about 3%.

The data obtained by all three methods on a given group of samples were combined and an analysis of variance was made for each group. The results of these analyses are shown in Table III. When all three methods were included in the analyses a significant difference between methods and a significant interaction (samples \times methods) was found for five out of the six groups of samples. The significant interactions were due mainly to the fact that method "C" gave consistently higher results than either methods "A" or "B" except on freshly prepared tablets. Hence, although method "C" had the same precision as methods "A" and "B" it did not have the same accuracy. Method "C" was concluded to be an unsatisfactory procedure since results obtained with the method appear to be markedly sample dependent. There were indications that method "C" measured certain steroid degradation products which were not measured by methods "A" and "B".

Separate analyses of variance were performed for each group of samples using only the data obtained by methods "A" and "B". The results of these analyses are shown in Table III also. In only one

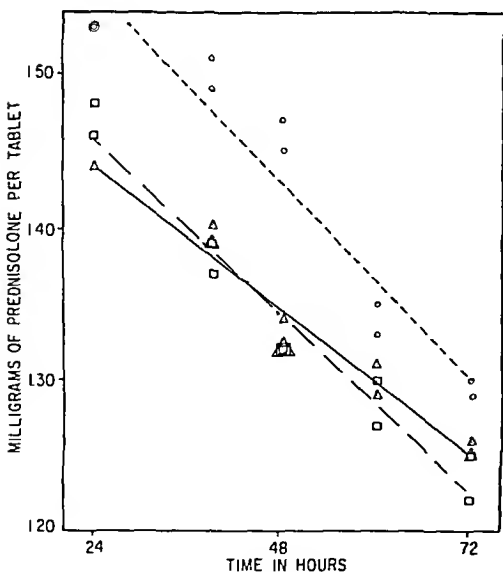


Fig 2—Plot of milligrams of prednisolone per tablet against time in hours for lot IA stored at 70° in sealed bottles with metal liner caps without Humi Caps. Δ —Method "A" \square —method "B", \circ —method "C".

² Dri Pax—A cloth bag enclosing approximately 3 Gm. of dried silica gel manufactured by Davison Chemical Company, Baltimore, Md.

TABLE II—TABULATION OF AVERAGES OF DUPLICATE ASSAYS OBTAINED BY THREE DIFFERENT METHODS ON ALIQUOTS OF THE SAME STABILITY SAMPLES

Potency: Milligrams of Prednisolone Per Tablet						
Methods	R T	R T + Humi Caps	40° Samples 40° + Humi Caps	17°	47° + Dri-Pax or Humi Caps	
Group III: Assays of Two Month Stability Samples of Lot I						
A	1 54		1 48	1 44		1 48 D-P
B	1 47		1 40	1 38		1 46
C	1 56		1 58	1 60		1 62
Group IV: Assays of Two Month Stability Samples of Lot II						
A	1 58		1 33	1 21		1 35 D-P
B	1 47		1 28	1 13		1 33
C	1 56		1 38	1 29		1 46
Group V: Assays of One Month Stability Samples of Lot IA						
A		1 55	1 42			1 50 H-C
B		1 51	1 45			1 53
C		1 53	1 55			1 66
Group VI: Assays of One Month Stability Samples of Lot IIA						
A		1.49	1 47			1 36 H-C
B		1.49	1 43			1 42
C		1 56	1 56			1 56

TABLE III—RESULTS OF ANALYSES OF VARIANCE OF ASSAY VALUES OBTAINED BY ALL THREE METHODS AND BY METHODS A AND B ONLY ON DIFFERENT GROUPS OF TABLETS

Data Analyzed	Analysis of All Three Methods		Analysis of Methods "A" & "B" Only	
	Among Samples	Among Methods ^c	Among Samples	Among Methods ^c
Group I ^d	N S ^a	0 001	0 05	N S
Group II ^e	0 001 ^b	0 001	0 001	0 05
Group III ^f	N S ^b	0 01	0 001	0 001
Group IV ^f	0 001 ^b	0 01	0 001	0 001
Group V ^f	N S ^b	N S	0 01	N S
Group VI ^f	N S ^b	0 05	N S ^b	0 05

^a N S = Not significant
^b Interaction (samples X methods) significant
^c between "methods" is due to the duplicate assays were made to spectrophotometric measurement and not from the powder aliquot to spectrophotometric measurement. No accurate estimate of the sampling error resulting from the use of different aliquots of ten crushed tablets of different tablet lots was made
^d See Fig 1
^e See Fig 2
^f Individual assay values from which averages in Table II calculated

out of six groups of samples was there a significant interaction (samples X methods) and this was obviously due to the difference in results obtained by methods "A" and "B" on the tablet Lot IIA stored for one month at 47°.

There was a significant difference between methods "A" and "B" when applied to four out of the six groups of samples. However, from a practical standpoint the difference in results obtained by methods "A" and "B" are very small in all six groups.³ The average deviation between results obtained by methods "A" and "B" is -0.02 mg of prednisolone per tablet with a range of -0.11 to +0.06 mg of prednisolone per 1.5-mg tablet (deviations taken as average of duplicate assays by method "B" minus average of duplicate assays by

method "A"). Expressing this in another way, one can say that potencies found by method "B" ranged from 93 to 104% with an average of 98.6% of the potencies found by method "A." It should be pointed out that such agreement between methods "A" and "B" may not apply to all tablet formulations or stability samples.

In Fig 3, the ordinate \bar{Y} , is the average potency found by duplicate assays with method "B" and the abscissa, \bar{X} , is the average potency found by duplicate assays with method "A." There are twenty-four such averages by each method. The "least squares" line, $\bar{Y} = 0.908 \bar{X} + 0.11$, calculated for these data was shown to have a slope not differing

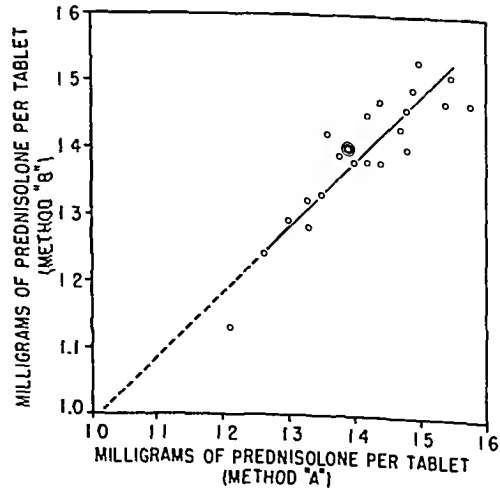


Fig. 3—Correlation of assay results obtained by method "B" with results obtained by method "A." The solid line is calculated from the average slope, 0.986

³ See also footnote "c," Table III.

significantly from unity and an intercept not differing significantly from zero. The solid line in Fig 3 represents the average slope, 0.986. Hence it may be concluded that results obtained by method "B" correlate well with results obtained by method "A."

In Fig 4, the ordinate, \bar{Y}' is the average potency found by duplicate assays with method "C" and the abscissa, \bar{X} , is the average potency found by duplicate assays with method "A." There are

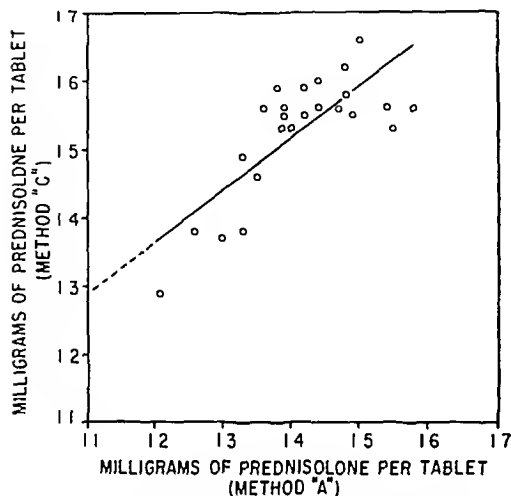


Fig 4—Correlation of assay results obtained by method "C" with results obtained by method "A." The line is calculated from the equation, $\hat{Y}' = 0.739\bar{X} + 0.48$ which is the fitted line obtained by means of the method outlined in reference (11) and by the method of "least squares."

twenty-four such averages by each method. The line shown on the plot was calculated from the equation $\hat{Y}' = 0.739\bar{X} + 0.48$ which is the fitted line obtained by means of the method of Bartlett (11) and also by the method of "least squares." It is obvious that method "C" does not give results correlating well with method "A."

SUMMARY AND CONCLUSIONS

1. A preliminary comparison of three methods of determining prednisolone in freshly made and aged tablets has been made.

2. A chloroform extraction-tetrazolium procedure and an alcohol extraction-tetrazolium procedure, are described in detail. The third method, a paper chromatographic procedure, has been described in detail in a previous communication.

3. The chloroform extraction-tetrazolium procedure gave comparable results to the paper chromatographic method for the samples checked. The former method, being less tedious, is the more desirable one for routine assays.

4. The alcohol extraction-tetrazolium procedure was found to be unsatisfactory and did not give results correlating well with the paper chromatographic method. The alcohol method gave high values for many of the stability samples although it compared well with the other methods when used for freshly prepared tablets.

5. The presence of a desiccant was shown to improve the stability of prednisolone in compressed tablets.

REFERENCES

- (1) Meister, P. D., Schlagel, C. A., Stafford, J. E., and Johnson, J. L., *This Journal*, 47, 576 (1958).
- (2) Mader, W. J., and Buck, R. R., *Anal. Chem.*, 24, 666 (1952).
- (3) Chen, C., Wheeler, J., and Tewell, H. E., Jr., *J. Lab. Clin. Med.*, 42, 749 (1953).
- (4) Meyer, A. S., and Lindberg, M. C., *Anal. Chem.*, 27, 813 (1955).
- (5) Nowaczynski, W., Goldner, M., and Genest, J., *J. Lab. Clin. Med.*, 45, 818 (1955).
- (6) Recknagel, R. O., and Litteria, M., *ibid.*, 48, 463 (1956).
- (7) Izzo, A. J., Burton, R. B., and Keutmann, E., *Federation Proc.*, 15, Part I, 280 (1956); *J. Clin. Endocrinol. and Metabolism*, 17, 889 (1957).
- (8) Schriebers, H., and Korus, W., *Naturwissenschaften*, 43, 517 (1956).
- (9) Youden, W. J., "Statistical Methods for Chemists," John Wiley & Sons, Inc., New York, 1951, (a) p. 24, (b) p. 45, (c) p. 49.
- (10) McArthur, D. S., Balch, F. I., White, W. H., and Anderson, J. S., *A*.
- (11) Bartlett, M. S.

A Study of the Titration of Organic Bases in Chlorobenzene with Acetous Perchloric Acid*

By M. PERNAROWSKI and D. W. BLACKBURN†

Potentiometric titrations can be carried out in chlorobenzene by using glass and sleeve-type calomel electrodes and a Beckman Model GS pH Meter. Acetous perchloric acid is the most suitable titrant. Galvanometer response is rapid and no erratic galvanometer needle deflections are noted when this titrant is used. The optimum concentration of titrant at the end point appears to be approximately 20 parts titrant for every 100 parts of solution. Bases as weak as caffeine can be determined in this solvent but only with a limited degree of accuracy. A mixed solvent containing 20 per cent glacial acetic acid in chlorobenzene can be used for the titration of organic bases insoluble in chlorobenzene. Bromophenol blue is a suitable indicator for use in chlorobenzene. Neutral red, brilliant green, and methyl red can also be used in this solvent but indicator changes are not as satisfactory as with bromophenol blue.

TWENTY-FIVE YEARS ago, Conant, Hall, and WERNER (1-3) showed that many substances which exhibit no basic properties in water behave as relatively strong bases in glacial acetic acid. The analytical significance of their observations was not immediately realized and the technique of acid-base titrations in nonaqueous solvents was not generally accepted until after the second world war. The distinct advantage of this method, as compared with volumetric procedures carried out in aqueous media, made it particularly suited to the quality control of pharmaceuticals. The application of acid-base titrations to the analysis of pharmaceutically important compounds has been adequately reviewed by Pifer, Wollish, and Schmall (4). Reviews of a more general nature have been published by Riddick (5-7) and Ballezo (8, 9).

Research in this field has followed the pattern laid down by Conant, Hall, and Werner. The "super acids" appeared to offer the ultimate in base intensification and solubilization. A large number of papers, therefore, appeared in the literature dealing with the analysis of various organic bases in glacial acetic acid. It soon became evident that other solvents were superior to glacial acetic acid both in solubilizing ability and in base-strengthening power. Pifer, Wollish, and Schmall (10) showed that the addition of aprotic solvents to glacial acetic acid greatly increased the magnitude of the potential break at the end point in the potentiometric titration of very weak bases with acetous perchloric acid. Fritz (11) reported that many solvents gave as good or better results than did glacial acetic acid.

Chlorobenzene can be classified as an inert or

aprotic solvent. Little ionization occurs in such solvents (8) and it is, therefore, an excellent medium for the reaction of basic compounds with acetous perchloric acid. The solvent, however, has not been extensively investigated. Fritz (11) reported the determination of several organic compounds in this solvent using either indicators or a glass and silver-silver chloride electrode combination to detect the end point of the titration. Rice, Zuffanti, and Luder (12) studied indicator color changes with "L-acids" in chlorobenzene. Deal, Weiss, and White (13) determined basic nitrogen compounds in asphalt by titration in a 50 per cent mixture of glacial acetic acid in chlorobenzene with acetous perchloric acid. They reported that potentiometric titration in chlorobenzene alone was not feasible.

EXPERIMENTAL

At the outset, it was necessary to establish proper conditions for potentiometric titration in chlorobenzene. Neither the glass and fiber-type calomel electrode combination nor the glass and silver-silver chloride electrode combination suggested by Fritz (11) were found to be suitable. Potentiometric titrations of several organic bases using these electrodes and a Beckman Model GS pH Meter were unsatisfactory because of erratic galvanometer response. Similarly, the gold and calomel electrode combination suggested by Zeidler (14) and Novak (15) was found to be unsatisfactory for use in chlorobenzene. A glass and glass sleeve-type calomel electrode combination showed satisfactory response during potentiometric titration of basic compounds in chlorobenzene.

Preliminary investigation of several titrants showed that acetous perchloric acid was the most suitable titrant for use in chlorobenzene. Perchloric acid in dioxane and perchloric acid in 20% glacial acetic acid in chlorobenzene were unsatisfactory because of erratic galvanometer response. Diphenyl phosphate, a titrant used by Davis and Hetzer (16) for titrations in aprotic solvents, was

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unsatisfactory because of its poor solubility in chlorobenzene

These exploratory potentiometric titrations of several organic bases in chlorobenzene established that satisfactory titration curves could be obtained using glass and sleeve type calomel electrodes, a Beckman Model GS pH Meter, and acetous perchloric acid as titrant. These observations confirm literature reports that the glass and sleeve-type calomel electrode combination and acetous perchloric acid are generally the most satisfactory electrode combination and titrant for use in alkali-metric titrations in nonaqueous media

Titration Assembly.—A Beckman Blue Label Glass Electrode No 1190-80 and a Beckman Sleeve-Type Calomel Electrode No 1170-71 were inserted into a Beckman Electrode Holder and placed in the solution being titrated. The Beckman Electrode Holder provided standard electrode spacing throughout the titrations. Decreasing this distance between electrodes did not appear to have any beneficial effect on the nature of the potentiometric titration. The electrodes were connected to a Beckman Model GS pH Meter, Serial No 97182, and the potentiometric titration was carried out with the sensitivity switch in position A and the pH Mv switch in position +Mv. During the titration, the acetous perchloric acid and the solution were mixed with a glass stirring rod. A magnetic stirrer may be used but extreme galvanometer deflection results which necessitate disconnection of the stirrer when millivolt readings are being taken.

Solvent Purification.—Approximately 10 Gm of anhydrous granular calcium chloride were added to 500 ml of chlorobenzene (Eastman reagent grade) and allowed to stand for twenty-four hours. The solvent was distilled from an all glass apparatus and the distillate collected when the boiling point became constant. The procedure was repeated and after the second distillation the solvent was considered ready for use. The observed boiling point was 129.5°. Literature values of 130.25–132° have been reported (17). Refractive index values agreed satisfactorily with the values reported in the literature.

Choice and Purity of Organic Bases Titrated.—Nine organic compounds, with pK_b values in water ranging from 4.35 to 14.00, were studied during the course of this investigation. In addition, salts of two organic bases and one salt of an organic acid were included in the list of compounds titrated during the investigation. The organic bases and salts chosen were atropine, strychnine, ephedrine, amine, cinchonine, amidopyrine, benzocaine, caffeine, theobromine, atropine sulfate, ephedrine sulfate, and potassium acid phthalate. All the organic bases were dried in an oven set at 100° or over phosphorous pentoxide depending on the melting point of the compound.

The melting points of the organic bases were determined using either a Purdue melting point bath or a Fisher-John melting point apparatus. If the melting point of the compound did not agree with the value reported in the literature, the organic base was recrystallized by dissolving in a minimal amount of alcohol and precipitating with an excess of distilled water. Atropine sulfate was recrystallized from dioxane.

Titrant.—The acetous perchloric acid used in this investigation was prepared and standardized according to the procedure given by Seaman and Allen (18). The acid was added to the solution of the organic base in chlorobenzene or 20% glacial acetic acid in chlorobenzene from a 50 ml buret calibrated to 0.02 ml. Increments of 0.02 ml were added to the solution in the end point region both in potentiometric and indicator titrations. No attempt was made to add less than 0.02 ml of titrant even though end point detection to 0.01 ml is possible in many acid-base titrations in nonaqueous media.

Titrations in Chlorobenzene.—One milliequivalent weight of the organic base was transferred quantitatively with the aid of chlorobenzene to a previously calibrated 100-ml volumetric flask. The solution was diluted to 100.0 ml with chlorobenzene, mixed, and 25.0 ml aliquots were measured from a buret for potentiometric titration with 0.0460 *N* acetous perchloric acid. The same procedure was used for indicator titrations in chlorobenzene.

This procedure provided a means of titrating 0.25 milliequivalent weight of the organic base without resorting to the weighing of semimicro quantities of material. Moreover, a direct comparison of all titration curves could be made since the ratio of solution to titrant was approximately the same in all titrations. Each titration required approximately 5.4 ml of acetous perchloric acid and the ratio of titrant to solution at the end point was, therefore, 21.6 to 100.

Titrations in 20% Glacial Acetic Acid in Chlorobenzene.—The samples were prepared for titration in a manner similar to that given above. One milliequivalent weight of the organic base was transferred to a 100 ml volumetric flask with the aid of 20.0 ml of glacial acetic acid and sufficient chlorobenzene was added to make the final volume 100.0 ml. Titrations were carried out on 50 ml aliquots of the solution. This, however, did not change the ratio of titrant to solution since both the volumes of titrant and solution were doubled.

Titrations were carried out in this mixed solvent system for two reasons.

(a) It has been reported in the literature that the addition of solvents of low dielectric constant to solutions of weak bases in minimal amounts of glacial acetic acid enhanced the potential change at the end point (10). This phenomenon occurs even when the glacial acetic acid content of the solution is further increased by the addition of acetous perchloric acid. Potentiometric titrations in 20% glacial acetic acid in chlorobenzene would confirm this observation.

(b) Many organic bases are readily soluble in glacial acetic acid. Since it was expected that several of the organic bases used in this investigation would be insoluble in chlorobenzene, some other method of solubilization had to be used that would retain a solvent system consisting mainly of chlorobenzene. Glacial acetic acid was found to be the most satisfactory solubilizing agent investigated. It provided a stable titration medium for potentiometric titration and, at the same time, solubilized the majority of the organic bases used in this investigation.

Other solubilizing agents were investigated in this phase of the work. It has been reported in the literature that morphine and ecodeine phosphate can be easily solubilized in chloroform by the addition of 1% and 5% phenol, respectively (19, 20). Addition of 5% phenol to the chlorobenzene was found to be unsuitable because of erratic galvanometer response during potentiometric titration. In addition, the solubility of the organic base was not materially increased.

DISCUSSION

The solubility of the organic bases used in this investigation in chlorobenzene or 20% glacial acetic acid in chlorobenzene was better than expected. Only alanine was found to be insoluble in both solvent systems. Theobromine and cinchonine were insoluble in chlorobenzene but, at the same time, their solubility in glacial acetic acid was poor. Heat was used to solubilize these bases in glacial acetic acid prior to the addition of chlorobenzene. The alkaloidal salts and the salt of the organic acid used in this investigation were solubilized in 20% glacial acetic acid in chlorobenzene without any difficulty.

On the basis of experimental observations, it would appear that the titrant should be approximately 0.05 *N* in order to carry out a satisfactory potentiometric titration. In addition, the ratio of titrant to solution at the end point should be approximately 20 to 100. The addition of larger quantities of titrant increased the stability of the solvent system during the potentiometric titration but, at the same time, decreases the value of the factor dE/dV at the end point. The ratio of 20 parts of titrant to 100 parts of solution is not critical. Several titrations were performed in which the titration at the end point was approximately 5 to 100. Although it is possible to carry out titrations at this level, the solvent system is not as stable as with higher titrant to solution ratios.

Typical titration curves for the weak base amidopyrine in chlorobenzene and in 20% glacial acetic acid in chlorobenzene are shown in Fig. 1.

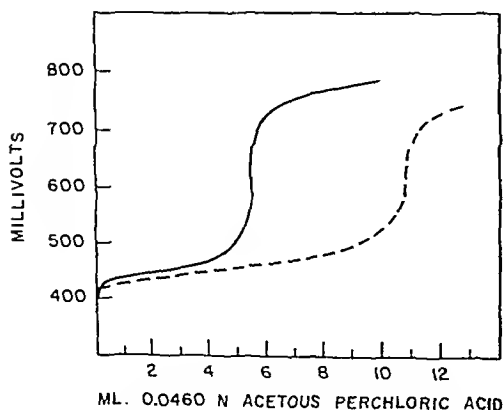


Fig. 1.—Titration curve for amidopyrine in chlorobenzene — and in 20% glacial acetic acid in chlorobenzene ---.

The titration curves for the stronger bases showed greater potential changes at the end point while the potential changes for the weaker bases were less pronounced. As an example, in chlorobenzene the value of the factor dE/dV at the end point for strychnine, a moderately strong base in nonaqueous media, was found to be approximately 4,000 for 0.02 ml. of titrant. Amidopyrine, a weaker base in nonaqueous media, showed a potential change of approximately 3,000 for 0.02 ml. of titrant. The value of the factor dE/dV at the end point for benzocaine, a very weak base, was found to be approximately 400 for 0.04 ml. of titrant.

In this respect, there does not appear to be any pattern for potential changes at the end point in chlorobenzene. Bases with pK_b values in water of less than 10 show essentially the same magnitude of change at the end point. The very weak bases, on the other hand, differ greatly from those with pK_b values in water of less than 10. For these very weak bases, the values of the factor dE/dV at the end point range from 2 to 10% of the values observed for the stronger bases. Those compounds with pK_b values in water of more than 10 can be determined only by using exacting techniques and careful millivolt measurements. Other solvents would be preferable for the determination of these compounds.

Each organic base was titrated in triplicate with acetous perchloric acid and the titration curves compared. The titration curves for the stronger bases were essentially identical. Variations were observed in the titration curves of the weaker bases. Either a shift of the titration curve upward or a decrease in millivolt readings after the end point region may be expected. These variations in the titration curves of any one compound do not interfere with the detection of the end point.

Perchlorate precipitates form during the titration of organic bases in chlorobenzene. During the titration of atropine, the solution turns murky but no true precipitate forms. Ephedrine does not form an insoluble perchlorate salt. These precipitates do not interfere with the titration of the organic compound. There is some evidence that the formation of crystalline precipitates during the titration aids in the detection of the indicator end points of very weak bases (21).

Bromophenol blue, methyl red, neutral red, brilliant green, and diazoamidobenzene were found to be suitable for the detection of the end point during the titration of bases stronger than amidopyrine. The color changes of these indicators at the end point are shown in Table I. Indicator color changes for bases having pK_b values in water

TABLE I.—INDICATOR COLOR CHANGES FOR STRONG BASES TITRATED IN CHLOROBENZENE WITH ACETOUS PERCHLORIC ACID

Indicator	Base Color	Acid Color
Bromophenol blue	Yellow ^a	Colorless
Neutral red	Orange ^a	Purple
Brilliant green	Green	Yellow-green
Methyl red	Orange	Red
Diazoamidobenzene	Yellow-green	Orange

^a The initial color of this indicator varies with the strength of the base.

of more than 10 were gradual and the end point could not be detected. Diazoamidobenzene is suitable only for the titration of atropine and ephedrine. The formation of perchlorate precipitates obscures the color change at the end point and overestimation of the organic base results. Of the five indicators investigated, bromophenol blue appears to be the most satisfactory indicator for alkalimetric titrations in chlorobenzene.

Bromophenol blue and neutral red exhibited unique characteristics in this solvent. The initial colors of these indicators in the solution of the organic base in chlorobenzene appeared to signal the strength of the compound. Addition of bromophenol blue to solutions of ephedrine or atropine in chlorobenzene resulted in the formation of a purple color. Solutions of strychnine in chlorobenzene exhibited rose-red colors when the indicator was added. Yellow colors resulted when the indicator was added to solutions of amidopyrine, benzocaine, or caffeine in chlorobenzene. The intensity of the yellow color, however, varied with the strength of the base. A bright yellow color was observed when amidopyrine was the organic base under investigation and pale yellow colors were observed with chlorobenzene solutions of caffeine and benzocaine. A similar phenomenon was observed with neutral red indicator. This indicator, however, did not distinguish between atropine, ephedrine, and strychnine. Solutions of these three bases all showed the same yellow color. Pink indicator colors were observed in chlorobenzene solutions of caffeine and benzocaine. Amidopyrine showed an initial color of intermediate hue.

The accuracy and precision of either indicator or potentiometric titrations in chlorobenzene is as good as that observed in the titration of strong bases in aqueous media. The per cent recoveries of the weighed amounts of the organic bases were satisfactory. Six potentiometric titrations were carried out on samples of atropine in chlorobenzene. At the 0.1 milliequivalent weight level, the per cent recovery of the alkaloid was 100.51% and the standard deviation was equal to 1.12%. At the 0.2 milliequivalent weight level, 99.71% of the alkaloid was recovered and the standard deviation was calculated to be 0.55%. This would indicate that the procedure gives more precise results when slightly larger quantities of the organic base are titrated.

All the organic bases, with the exception of alanine, were also titrated in 20% glacial acetic acid in chlorobenzene. From the potentiometric titrations performed in this solvent system, three conclusions may be reached:

(a) The addition of large amounts of glacial acetic acid to chlorobenzene are to be avoided. In all cases, the magnitude of the potential break at the end point was sharply decreased from the value observed in chlorobenzene alone. The magnitude of the potential break at the end point for all compounds titrated in this investigation both in chlorobenzene and in 20% glacial acetic acid in chlorobenzene are shown in Fig. 2. No absolute values are reported but only relative values based on 0.1 ml. of titrant. As an example, if the value of the factor dE/dV at the end point was 4,000 for 0.02 ml. of titrant, the value reported in this figure would be $4,000 \times 0.10/0.02$ or 20,000.

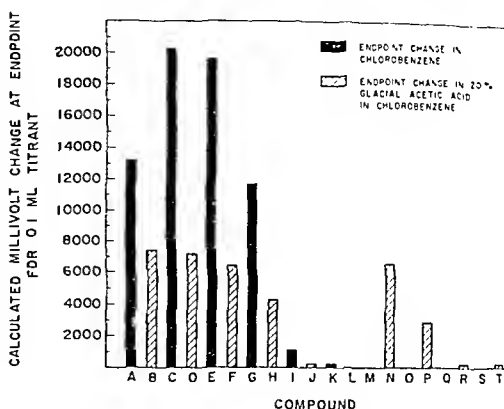


Fig. 2.—Relative changes at end point for atropine (AB), strychnine (CD), ephedrine (EF), amidopyrine (GH), benzocaine (IJ), caffeine (KL), potassium acid phthalate (MN), cinchonine (OP), ephedrine sulfate (QR), and atropine sulfate (ST). MOQS: insoluble in chlorobenzene.

(b) The titrations carried out in 20% glacial acetic acid in chlorobenzene confirm literature reports that the magnitude of the potential break at the end point is increased by the addition of large amounts of aprotic solvents to minimal amounts of glacial acetic acid (10).

(c) Alkaloidal salts are insoluble in chlorobenzene. They are, however, soluble in 20% glacial acetic acid in chlorobenzene and can be determined accurately by titration with acetic perchloric acid. The determination of atropine sulfate and ephedrine sulfate have been previously reported in the literature (22, 23). From personal observations and from literature reports (24), it would appear that the reaction of sulfates with perchloric acid in a glacial acetic acid medium are not stoichiometric. By the addition of chlorobenzene to a glacial acetic acid, stoichiometric results can be obtained. Potential breaks for the two alkaloidal salts are not too great, but the end point can be determined easily.

From this discussion of the titration of organic bases in chlorobenzene, it is evident that the solvent is suitable for use in alkalimetric titrations with acetic perchloric acid. Either potentiometric or indicator methods can be used to detect the end point. Very weak bases can be titrated in this solvent but only with a limited degree of accuracy. Determination of such bases, therefore, would be better carried out in other organic solvents.

REFERENCES

- (1) Hall, N. F., and Conant, J. B., *J. Am. Chem. Soc.*, **49**, 3047 (1927).
- (2) Conant, J. B., and Hall, N. F., *ibid.*, **49**, 3062 (1927).
- (3) Hall, N. F., and Werner, T. H., *ibid.*, **50**, 2367 (1928).
- (4) Pifer, C. W., Wollish, E. G., and Schmalt, M., *THIS JOURNAL*, **42**, 509 (1953).
- (5) Riddick, J. A., *Anal. Chem.*, **24**, 41 (1952).
- (6) Riddick, J. A., *ibid.*, **26**, 77 (1954).
- (7) Riddick, J. A., *ibid.*, **28**, 679 (1956).
- (8) Balczon, H., *Mitt. chem. Forschungs-inst. Wirtsch. Österr.*, **7**, 104 (1954).
- (9) Balczon, H., *ibid.*, **8**, 37 (1954).
- (10) Pifer, C. W., Wollish, E. G., and Schmalt, M., *Anal. Chem.*, **25**, 310 (1953).
- (11) Fritz, J. S., *ibid.*, **22**, 578 (1950).
- (12) Rice, R. V., Zuffanti, S., and Luder, W. F., *ibid.*, **24**, 1022 (1952).

- (13) Deal, V. Z., Weiss, F. T., and White, T. T., *ibid.*, 25, 426(1953).
 (14) Zeidler, H. Z., *ibid.*, 146, 251(1955); through *Chem. Abstr.*, 49, 12175i(1955).
 (15) Novak, V., *Chem. listy*, 49, 848(1955); through *Chem. Abstr.*, 49, 13011a(1955).
 (16) Davis, M. M., and Hetzer, H. B., *J. Research Natl. Bur. Standards*, 54, 309(1955).
 (17) Riddick, J. A., and Toops, E. E., "Organic Solvents," Interscience Publishers, Inc., N. Y., 1955, p. 408.
 (18) Seaman, W., and Allen, E., *Anal. Chem.*, 23, 592(1951).

- (19) Pernaowski, M., Chatten, L. G., and Levi, L., *This Journal*, 43, 746(1954).
 (20) Dietzel, R., and Paul, W., *Arch. Pharm.*, 276, 408(1938).
 (21) Poulos, A., *Anal. Chem.*, 24, 1858(1952).
 (22) Arkel, C. G. van, and Kroonenberg, J., *Pharm. Weekblad.*, 87, 137(1952); through *Chem. Abstr.*, 46, 7450c(1952).
 (23) Pifer, C. W., and Wollish, E. G., *Anal. Chem.*, 24, 519(1952).
 (24) Kashima, T., Asahina, H., and Shiuehi, Y., *J. Pharm. Soc. Japan*, 75, 329(1955); through *Chem. Abstr.*, 49, 9885a(1955).

Chronic Oral Toxicity of Aerated Silica Gel*

By CHANDRAKANT H. DESAI, ALLAN BURKMAN, and RUPERT SALISBURY

It would appear that aerated silica gel of the type used is not entirely inert in rats since there appears to be a significant change in the body associated with its continued administration. A comprehensive investigation of chronic oral toxicity of aerated silica gel seems necessary before its oral use can be approved.

IN THE COURSE of a series of investigations testing the use of a relatively inert, insoluble material in the prevention of caking in pharmaceutical suspensions, aerated silica gel¹ showed some promise and chronic oral toxicity tests were undertaken to determine the feasibility of oral administration of large amounts of aerated silica gel.

Dried silica gel is a tasteless, odorless, relatively inert powder which has an enormous surface area (the material used was of a particularly fine particle size, from 3 to 5 microns in diameter) and is claimed to be capable of adsorbing toxins; it was used for this purpose locally in severe burn cases and internally in the treatment of certain types of gastroenteritis (1).

Intravenously administered injections of colloidal silica caused rapid death in mice according to Dale and King (2). Injection of 5 mg. of finely powdered silica gel was injected into each renal artery of rats with a resulting hypertension of many months' duration because of diffuse intrarenal fibrous tissue action (3).

In studying the toxicity of silica in various textbooks of toxicology (4, 5) it becomes apparent that the greatest interest has been in industrial silicosis due to the mechanical irritation produced

by inhalation rather than by ingestion of the material. Since aerated silica gel has properties which have possibilities for internal application and since no work has been done on its oral chronic toxicity, this study was undertaken to evaluate the chronic oral toxicity of aerated silica gel.

EXPERIMENTAL

Male and female, Sprague-Dawley, young adult rats were utilized in the study. Throughout the experiment they were fed Purina Laboratory Chow and water *ad libitum*. Test group I consisting of 12 rats received a daily oral dose of aerated silica gel equivalent to 5.71 mg./Kg. for a period of eight weeks. Test group II (12 rats) received a daily oral dose of aerated silica gel equivalent to 17.14 mg./Kg. A control group of 12 rats received a dose of methylcellulose equivalent to 5.71 mg./Kg.

The 36 rats used were approximately of the same age and weight. Oral administration was by stomach tube and syringe. All the rats were weighed initially and then weekly. Doses were calculated on a weekly basis and administered daily.

RESULTS AND DISCUSSION

The eight weeks of daily oral administration of aerated silica gel in different concentrations and the use of methylcellulose as a control did not produce a single untoward reaction in the experimental animals. All remained normal with no signs of abnormality. During the eight week experiment it was observed that the rate of gain in weight in the experimental groups was not as great as that in the control groups (Fig. 1). Due to the rather small groups of animals used and in view of the more significant pathological findings no significance is attached to the change in rate of gain in weight at this time.

Post-mortem examination revealed abnormal larger nodules on the intestine of the test rats and not on the control group. There were more nodules in the test rats which received the larger amounts of aerated silica gel. All other factors were quite normal.

* Received February 2, 1958, from the College of Pharmacy, The Ohio State University, Columbus.

Abstract of a portion of a dissertation presented to the Graduate School of The Ohio State University by Chandrakant H. Desai in partial fulfillment of the requirements for the degree of Master of Science.

¹ Kindly supplied by the Monsanto Chemical Co. under the trade name, Santocel C.

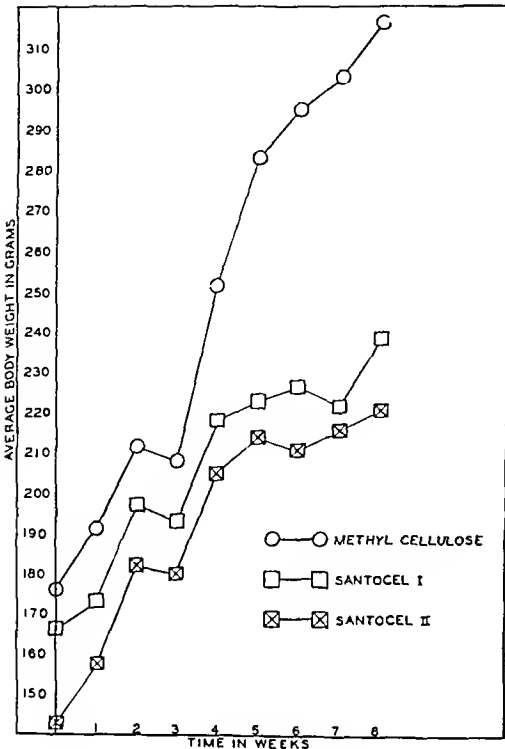


Fig 1.—Growth curves

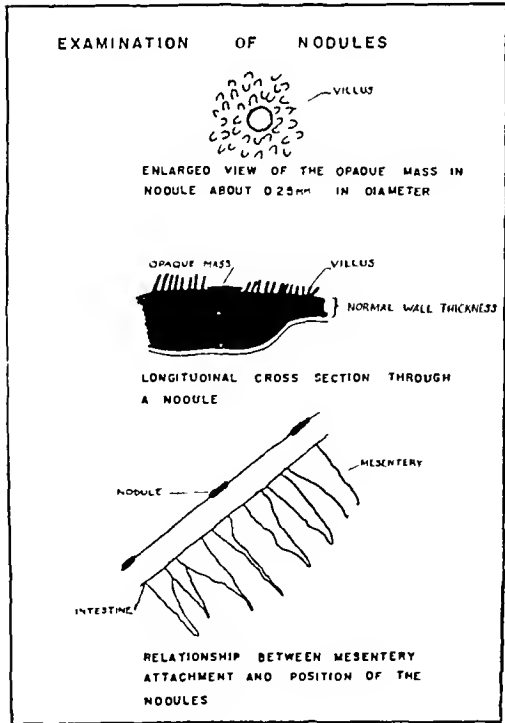


Figure 2.

Gross Description of Intestinal Nodules.—Palpable masses of firm tissue were on the external surface opposing the area of mesentery attachment of the gut wall (Fig. 2). Numerous dense opaque disk-shaped masses devoid of villi characterized the nodule when observed within the intestinal lumen (Fig. 3). They were externally similar to the tubercle-like lesions of tuberculosis or neoplastic disease; lesions larger than those of tuberculosis but less diffuse were limited only to the gut wall

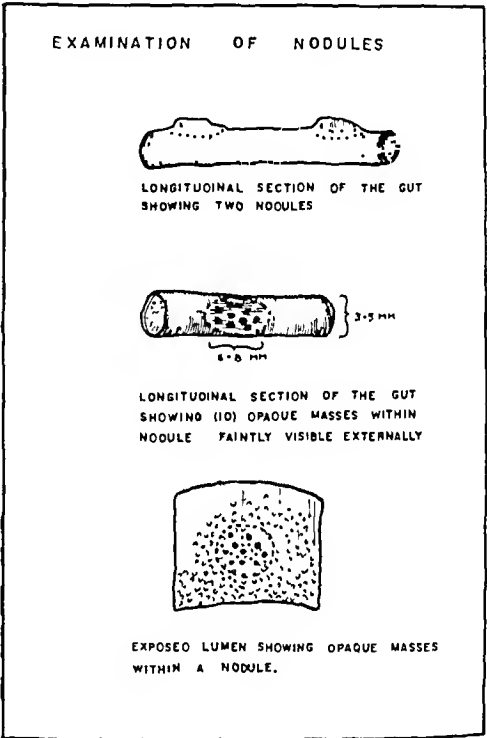


Figure 3.

Microscopic Description.—The major portion of the nodule consists of masses of lymphoid connective tissue.

Etiology of Nodules.—The position of the nodules and their etiologic characteristics suggest that they are very much enlarged Payer's patches. The enlargement in turn is a typical response to foreign body invasion, possibly caused by very small insoluble silica gel particles imbedded in tissue with the formation of nodules through foreign body reaction.

REFERENCES

(1) Osol-Farrar, "United States Dispensatory," 25th ed. J. B. Lippincott Co., Philadelphia, 1935, p. 1848.
(2) Dale, C. J., and King, E. J., *Arch. Ind. Hyg. Occup. Med.* 7, 479 (1953).
(3) Campbell, M., *Chem. Abstr.*, 48, 10,200 (1954).
(4) Von Oettingen, "Poisoning," Paul B. Hoeber, Inc., New York, 1954, p. 459.
(5) Thiemes, Clinton H., "Clinical Toxicology," Lea & Febiger, Philadelphia, 1940, p. 177.

The Effect of Some Antihistamines upon the Respiration of Rat Heart Homogenates*

By DORIS E. McKERSIE and HAROLD C. HEIM

Conventional manometric techniques were used to investigate the effects of some antihistamines upon the respiration of rat heart homogenates. The study was undertaken because it has been reported that these drugs exert effects on cardiac arrhythmias but little is known relative to the mechanisms by which these effects are produced. The antihistamines which were studied were promethazine HCl, phenindamine tartrate, methapyrilene HCl, antazoline HCl, diphenhydramine HCl, trippellenamine HCl, prophepyridamine maleate, and pyrilamine maleate. Each of these drugs, in 0.007 *M* concentration, inhibited endogenous respiration as well as respiration in the presence of glucose or pyruvate. Both promethazine HCl and phenindamine tartrate markedly inhibited respiration in the presence of succinate. None of the other antihistamines included in the study produced any significant effect upon respiration in the presence of succinate. The effects elicited by the antihistamines could not be significantly altered by the addition of histamine to the Warburg vessels.

SEVERAL INVESTIGATORS have reported that certain antihistamine drugs exert cardio-depressant activity. It has been reported (1) that diphenhydramine exerts an effect similar to that of quinidine in converting an auricular fibrillation to a normal sinus rhythm in dogs previously sensitized to epinephrine by the administration of chloroform. It has been shown (2) that some antihistamines, at rather high dosage levels, were effective in preventing ventricular arrhythmias induced in rats by the administration of calcium chloride and that in even higher dosages they were effective in overcoming the arrhythmias induced by the previous administration of aconitine. Reports of other studies have appeared in which it has been shown that diphenhydramine is more effective than quinidine in abolishing the fibrillation produced in the dog heart by the administration of acetylcholine (3).

It appears that little attention has been devoted to the effects of the antihistamines on respiratory processes of the heart. In view of the fact that several reports indicate that the antihistamines exhibit some cardiodepressant activity it seemed of interest to study the effects elicited upon the respiration of heart homogenates by certain drugs in this group.

EXPERIMENTAL

The hearts from healthy, adult Sprague-Dawley rats of either sex and weighing between 200 and 250 Gm. were used in all experiments. The animals were killed by a blow to the head after which the hearts were immediately removed and placed in a beaker of ice-cold isotonic sucrose (0.25 *M*) solution. Each heart was allowed to beat for a few seconds in the isotonic sucrose solution in order to remove as much blood as possible. The hearts were then cut

open and blotted dry on filter paper after which they were weighed and placed in a glass homogenizer containing cold isotonic sucrose solution. The homogenates were prepared so that each ml. of homogenate contained 160 mg. fresh heart tissue. No longer than twenty minutes elapsed from the time the animals were killed to the start of the experiment. Oxygen consumption was determined by the direct method of Warburg. When endogenous respiration was being studied 1.9 ml. of buffer, 0.4 ml. of the antihistamine solution or 0.4 ml. of distilled water, and 0.5 ml. of the freshly prepared homogenate were added to the main compartment of Warburg vessels of approximately 15-ml. capacity. The center well contained 0.2 ml. of 10% potassium hydroxide and a pleated strip of filter paper. When respiration in the presence of substrates was being studied 1.6 ml. of buffer and 0.3 ml. of the particular substrate were added to the main compartment in place of 1.9 ml. of buffer. In all experiments the total volume of the flask contents was 3.0 ml. The buffer used in all experiments was 0.1 *M* phosphate, pH 7.3.

The antihistamines under investigation were pyrilamine maleate (Neoantergan), prophepyridamine maleate (Trimeton), trippellenamine HCl (Pyrribenzamine), diphenhydramine HCl (Benadryl), promethazine HCl (Phenergan), phenindamine tartrate (Thephorin), methapyrilene HCl (Thenylene), and antazoline HCl (Antistine). The substrates studied were pyruvate, succinate, and glucose. The final concentration of each substrate was 0.01 *M* and the final concentration of each antihistamine was 0.007 *M*. Eight hearts were used for each experiment and duplicate flasks were run in every instance.

After a ten minute equilibration period the manometers were closed and readings were taken after an hour had elapsed. The temperature of the bath was 37° and the gas phase was air. All pH determinations were made with a glass electrode instrument.

Standard errors were calculated from the equation

$$\epsilon = \sqrt{\frac{\sum d^2}{n(n-1)}} \text{ and if the condition } M_1 - M_2 >$$

$2\sqrt{\epsilon_1^2 + \epsilon_2^2}$ was found to be true, the results were considered to be significant.

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TABLE I.

Substrate	Antihistamine	mm. ³ O ₂ Uptake in 60 Min./100 mg. Fresh Tissue	Inhibition (-) or Stimulation (+), %
None	None	23 ± 0.85 ^a	
None	Diphenhydramine HCl	13 ± 0.80	-44
Glucose	None	15 ± 0.75	
Glucose	Diphenhydramine HCl	10 ± 0.87	-33
Pyruvate	None	31 ± 0.94	
Pyruvate	Diphenhydramine HCl	14 ± 0.55	-55
Succinate	None	401 ± 5.89	
Succinate	Diphenhydramine HCl	387 ± 4.72	+4
None	None	21 ± 0.91	
None	Triptellenamine HCl	13 ± 0.74	-42
Glucose	None	18 ± 0.84	
Glucose	Triptellenamine HCl	11 ± 0.64	-39
Pyruvate	None	33 ± 1.18	
Pyruvate	Triptellenamine HCl	11 ± 0.72	-67
Succinate	None	432 ± 3.88	
Succinate	Triptellenamine HCl	438 ± 4.04	+1
None	None	18 ± 1.13	
None	Propenpyridamine Maleate	10 ± 0.80	-45
Glucose	None	18 ± 1.13	
Glucose	Propenpyridamine Maleate	13 ± 1.09	-38
Pyruvate	None	33 ± 1.24	
Pyruvate	Propenpyridamine Maleate	15 ± 1.22	-55
Succinate	None	441 ± 5.49	
Succinate	Propenpyridamine Maleate	436 ± 5.28	-1
None	None	21 ± 0.89	
None	Pyrilamine Maleate	11 ± 0.66	-48
Glucose	None	23 ± 0.99	
Glucose	Pyrilamine Maleate	11 ± 0.69	-52
Pyruvate	None	33 ± 1.15	
Pyruvate	Pyrilamine Maleate	11 ± 0.66	-67
Succinate	None	414 ± 4.85	
Succinate	Pyrilamine Maleate	444 ± 8.06	+7
None	None	21 ± 1.07	
None	Promethazine HCl	11 ± 1.19	-48
Glucose	None	21 ± 0.74	
Glucose	Promethazine HCl	13 ± 0.98	-42
Pyruvate	None	34 ± 1.25	
Pyruvate	Promethazine HCl	13 ± 1.09	-62
Succinate	None	431 ± 3.73	
Succinate	Promethazine HCl	11 ± 1.09	-97
None	None	21 ± 1.14	
None	Phenindamine Tartrate	11 ± 0.95	-48
Glucose	None	25 ± 1.24	
Glucose	Phenindamine Tartrate	15 ± 1.03	-40
Pyruvate	None	35 ± 0.92	
Pyruvate	Phenindamine Tartrate	20 ± 1.42	-43
Succinate	None	414 ± 3.74	
Succinate	Phenindamine Tartrate	24 ± 2.08	-94
None	None	24 ± 1.32	
None	Methapyrilene HCl	13 ± 0.77	-46
Glucose	None	19 ± 0.89	
Glucose	Methapyrilene HCl	13 ± 0.61	-32
Pyruvate	None	36 ± 1.68	
Pyruvate	Methapyrilene HCl	14 ± 1.19	-61
Succinate	None	414 ± 5.40	
Succinate	Methapyrilene HCl	433 ± 6.25	+5
None	None	23 ± 1.48	
None	Antazoline HCl	14 ± 0.62	-39
Glucose	None	25 ± 1.27	
Glucose	Antazoline HCl	15 ± 1.37	-40
Pyruvate	None	38 ± 2.10	
Pyruvate	Antazoline HCl	16 ± 1.15	-58
Succinate	None	409 ± 5.94	
Succinate	Antazoline HCl	431 ± 7.11	+5

^a Standard error.

RESULTS AND DISCUSSION

The effects elicited by the antihistamines upon the endogenous respiration and upon respiration in the presence of glucose, pyruvate, and succinate are shown in the Table. The endogenous respiration and respiration in the presence of glucose or pyruvate was significantly inhibited by all the antihistamines included in this study. In general, the greatest inhibition was produced with pyruvate as the substrate. With succinate as the substrate six of the antihistamines produced only an insignificant change in respiration which, in some instances, was a slight inhibition and in others a slight stimulation. With two of the antihistamines, however, (phenindamine tartrate and promethazine HCl) respiration in the presence of succinate was very markedly inhibited. Both phenindamine and promethazine differ structurally to a considerable degree from the other antihistamines in that promethazine contains a phenothiazine nucleus while phenindamine may be thought of as a phenylindene derivative with a dimethylamino-ethyl side chain which has been cyclized to form a tetrahydropyridine ring.

Because the antihistamines are thought to elicit their principal therapeutic effects by blocking histamine from cellular receptor sites, it was decided to study the effect produced on respiration of the homogenates in the presence of both histamine and the antihistamines. It was found that the effects produced by the antihistamines were not reversed by histamine. Histamine, at a final concentration of 0.005 *M*, produced a slight augmentation of oxygen uptake in the presence of all three substrates and also seemed to produce a somewhat more significant stimulation of the endogenous respiration. When histamine and the antihistamines were both added to the homogenates, however, the respiration was inhibited to essentially the same degree as when the histamine was omitted. Respiration in the presence of succinate was not inhibited by a combination of histamine and antihistamine except in those flasks to which phenindamine or promethazine were added. In this latter instance the inhibition of succinate oxidation was the same whether or not histamine was added to the flasks. These results would indicate that the effects of the antihistamines on the respiration of rat heart is mediated through mechanisms other than those involving histamine.

In a previous study (4) it was shown that emetine, a cardiotoxic agent, inhibited respiration of heart homogenates both in the presence and in the absence of glucose or pyruvate, and that emetine stimulated respiration in the presence of succinate. The effect of the antihistamines upon respiration of heart, *in vitro*, seems to be similar to some extent to the effects produced by emetine, yet the administration of the antihistamines does not evoke the cardiotoxic effects which often follow the prolonged administration of emetine (5). Emetine, however, is a cumula-

tive drug while the antihistamines are rather rapidly metabolized or excreted (6).

It would seem conceivable that the relatively mild cardiodepressant effects elicited by some of the antihistamines might be mediated through an inhibition of the oxidation of metabolites other than succinate, thereby increasing the negative inotropic effect of succinate upon the myocardium. It has been reported (7) that succinate elicits a strong negative inotropic effect upon guinea pig heart slices, and that after poisoning with chlorobutanol or pentobarbital the heart slices were even more susceptible to this effect. Other investigators (8) have reported that, under conditions of reduced oxygen tension, succinate markedly increases the rate of oxygen consumption of dog myocardium and that succinate competes with other metabolites for oxygen, resulting in a lower rate of oxidation of the other normally occurring metabolites. The energy produced by the oxidation of succinate is thought to be incapable of increasing the rates of other metabolic processes which were depressed under conditions of low oxygen tension.

SUMMARY

1. The effects produced by eight different antihistamines upon the respiration of rat heart homogenates has been studied.

2. All the antihistamines studied produced inhibition of endogenous respiration and of respiration in the presence of glucose or pyruvate. In general, inhibition was greatest with pyruvate as the substrate.

3. Six of the antihistamines produced little, if any, effect on respiration in the presence of succinate. Two of the antihistamines (promethazine HCl and phenindamine tartrate) produced a very marked inhibition of respiration in the presence of succinate.

4. The effects on respiration were not significantly altered by the addition of histamine.

REFERENCES

- (1) McCawley, E., Weston, G., and David, N., *J. Pharm. Exptl. Therap.*, 102, 250 (1951).
- (2) Mulinow, M., Batle, F., and Malamud, B., *Arch. inter. Pharmacodyn.*, 102, 55 (1955).
- (3) Schallek, W., *ibid.*, 105, 221 (1956).
- (4) Deitrich, R., and Heim, H., *THIS JOURNAL*, 45, 562 (1955).
- (5) Gimble, A., Davison, C., and Smith, P., *J. Pharm. Exptl. Therap.*, 94, 431 (1948).
- (6) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," 2nd ed., The Macmillan Co., New York, 1955, p. 659.
- (7) Wollenberger, A., and Yaffe, S. J., *Proc. Soc. Exptl. Biol. Med.*, 75, 838 (1950).
- (8) Furchtgott, R., and Shorr, E., *J. Biol. Chem.*, 175, 201 (1948).

Enzymatic Decomposition of Digitalis Glycosides II*

By OLE GISVOLD

THE RESULTS of previous investigations (1) in our laboratories have shown that when the fresh leaves of *Digitalis lanata*, *Digitalis lutea*, and *Digitalis ferruginea* were disintegrated quickly in the presence of water or dilute methanol or ethanol, heated for a brief period and then filtered, we were able to isolate or detect the desglucoglycosides such as acetyldigoxin, acetyldigitoxin, digitoxin, digoxin, and gitoxin. Apparently this technique leads to the ready enzymatic hydrolysis of the terminal glucose residue found in the native glycosides. These studies have been extended in a qualitative way to the following digitalis species, i. e., *Digitalis purpurea*, *Digitalis mertonensis*, *Digitalis amandiana*, and *Digitalis siberica*. In all the digitalis species investigated the desglucoglycosides were obtained. In some cases this technique led to the ready isolation of the glycosides in a crystalline state. It should be recorded that in a model experiment, adjustment of the water or aqueous methanol or ethanol to a pH of 7 with a phosphate buffer did not alter the rapid enzymatic removal of the terminal glucose residue of certain digitalis glycosides.

Stoll's (2) enzyme inhibiting technique when applied to the detection of the native digitalis glycosides is time consuming and the final preparation is contaminated with many pigments. A greatly simplified technique has been developed which in my hands led to the ready detection of the native glycosides. It differed only from the brief enzymatic studies in that the fresh leaves were first boiled for five minutes in either water, dilute aqueous methanol or ethanol, phosphate buffered (pH 7) water or phosphate buffered (pH 7) dilute aqueous methanol or ethanol. Boiling for short periods of time apparently inactivates the carbohydrase or carbohydrases that enzymatically cleave the terminal glucose residue present in the native glycosides. In all the digitalis species investigated, Stoll's native glycosides could be detected. Buffered solutions were used in order to minimize the possibility of the hydrolysis of the glycosides. This, because the aqueous extracts of the fresh leaves of the digitalis species are below a pH of seven and the digitalis glycosides that have a 2-desoxy sugar directly attached to the C₃-OH group of the aglycone are sensitive to acid hydrolysis even in the cold.

The results of my investigations serve to emphasize further that the recording of the nature of the glycosides found in a given digitalis species should always be accompanied by an exact history of the plant material that was used. It becomes very clear that for isolation purposes of the cardiac glycosides found in the digitalis species the method of handling, i. e., collection, storage, etc., and techniques of extraction determine to a great degree the products that will be obtained. These factors also are extremely important when the whole leaf or extracts thereof are used for oral purposes. This because the amount of absorption that is obtained by the oral route is to a great degree dependent upon the composition of the glycosides that are present, i. e., lanatoside C versus digoxin or acetyldigoxin or purpurea glycoside-A versus digitoxin or more strikingly gitoxin versus gitoxin, etc.

A new solvent system (System II) has been developed that readily separates some of the native glycosides. It can be used by the ascending or descending techniques. Separations can be made in two to twenty-four hours depending on the composition of the glycosides and the degree of separation desired. This system also can be used to good advantage with the desglucosides. Systems I and II give good separation of the genins which previously (3) has been accomplished by another system on formamide impregnated paper. Systems requiring equilibration that will separate the genins also have been reported (4).

EXPERIMENTAL

Paper Chromatography.—Whatman No. 1 paper in sizes approximately 14 cm. x 26 cm. were used in the ascending technique and those used in the descending technique were 14 cm. x 38 cm.

Solvent System I.—This system is the same as that previously described (5). It consists of methyl isobutyl ketone-isopropyl ether, 100:25 (by volume), saturated with formamide. The stationary phase, formamide, was applied to the paper via a 30% solution in acetone. No equilibration is necessary.

Solvent System II.—This system was prepared from methyl isobutyl ketone, isopropyl ether, tetrahydrofuran, and formamide in the ratio of 40:10:15:15. The stationary phase is the same as that used in solvent system II. No equilibration is necessary.

The reagents that were used in the paper chromatographic studies were purified as previously described

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(1). Isopropyl ether reagent grade was passed through a column of aluminum oxide to remove the peroxides. Tetrahydrofuran also was passed through the same kind of column and then distilled and the fraction boiling at 65° was collected and used. The positions of the glycosides on the paper were detected by the use of the Raymond reagent (6) or by Jensen's trichloroacetic acid reagent (7). Each of these reagents has its advantages and disadvantages. The former is much less sensitive than the latter. The latter will show bright blue fluorescence with compounds such as gitoxin and other substances in concentrations that are not readily detected by the Raymond reagent. Furthermore a blue fluorescing substance may have the same R_f value as some other glycoside and thus completely mask a yellow or some other color that might be present. I have shown the presence of some substances in some of the species of digitalis that give a red color in the sulfuric acid layer in the Keller-Kiliani color reaction that do not have digitoxose and do not give a Raymond reaction on paper but do give a strong blue fluorescence with the trichloroacetic acid reagent. These substances apparently are not saponins.

Extracts from Brief Enzymatic Treatment.—Approximately 600 to 800-Gm. lots of the fresh leaves of each of the following digitalis species; *purpurea*, *siberica*, *ferruginea*, *mertonensis*, and *amandiana* were collected in the fall of 1958. These were treated as previously described using approximately 15% aqueous methanol as the solvent to obtain the primary extracts. Each of these extracts was extracted with three successive portions of a mixture of ether and methylene chloride (in a ratio of 3 to 1) and labeled extract A. This was followed by three successive extractions with ethyl acetate which when combined was labeled extract B. The above extracts were examined by paper chromatography.

The results of the studies recorded in this paper are directed toward the identification of those digitalis glycosides that occur in major amounts. Figure 1 is a typical chromatogram that was obtained from extracts A with solvent system I and the Raymond reagent. Figure 2 is a typical chromatogram that was obtained from extracts A with solvent system I and the trichloroacetic acid reagent. These two figures in part, substantiate the preceding discussion. This can be further illustrated by Fig. 3 in which special care was taken to place the same amounts at the starting line. The chromatogram before spraying with the reagents was cut longitudinally along the dotted lines indicated in Fig. 3.

Extracts B when examined paper chromatographically using solvent systems I and II showed the absence of the desglucoglycosides. Some of these extracts showed the presence of glycosides that moved on paper in the proximity of lanatoside C. No further extensive studies were carried out on these extracts at this time.

Isolation of the Crystalline Glycosides.—The following general procedure was used. The solid material that was obtained from extract A was dissolved in about 20 cc. of methylene dichloride and then diluted with 60 cc. of ether. This mixture was extracted quickly with two to four successive small portions of 1% aqueous sodium hydroxide. The organic layer was then washed once with 1%

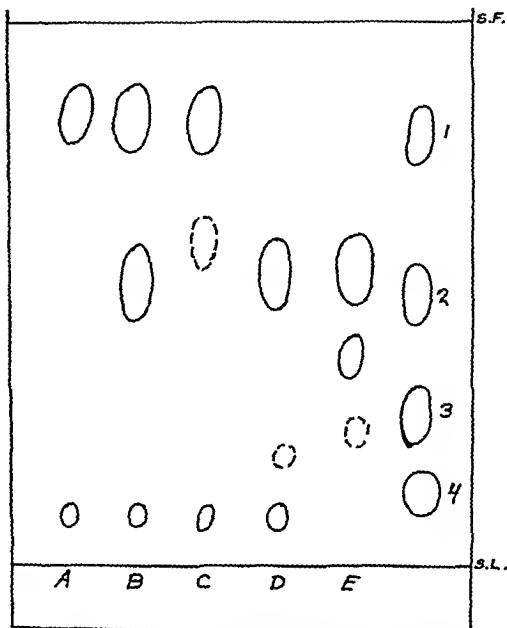


Fig. 1.—Solvent system I; S.F. = solvent front; S.L. = starting line; 1 = acetyldigitoxin; 2 = digitoxin; 3 = acetyldigoxin; 4 = digoxin; A = *Digitalis ferruginea*; B = *Digitalis amandiana*; C = *Digitalis siberica*; D = *Digitalis mertonensis*; E = *Digitalis purpurea*. Raymond reagent used to locate the glycosides.

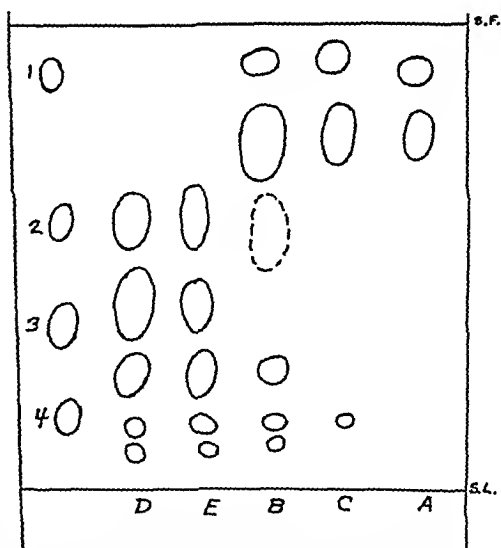


Fig. 2.—Same as that for Fig. 1 except trichloroacetic acid was used to locate the glycosides.

aqueous acetic acid and lastly once with water. At this point, in some cases, glycosidic material separated rather quickly and was subsequently identified as gitoxin, acetylgitoxin, or unknown glycosidic material depending on the species of plant under examination. The solvent was removed and the residue digested several times with petroleum ether.

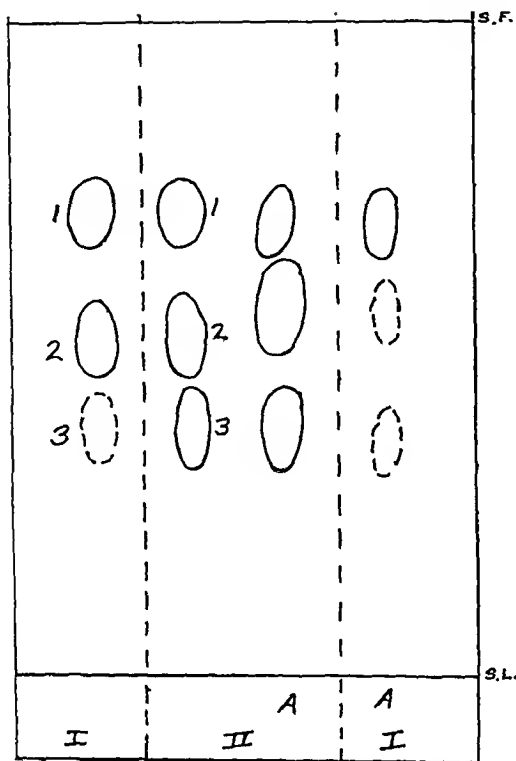


Fig. 3.—Solvent system I; S.F. = solvent front; S.L. = starting line. 1 = Digitoxin; 2 = acetyldigitoxin; 3 = gitoxin (as an impurity in digitoxin). A = First crop of crystals that were obtained from *Digitalis mertonensis*. I = Portion sprayed with the Raymond reagents; II = Portion sprayed with trichloroacetic acid reagent.

The residue was then dissolved in a small quantity of methylene dichloride and carefully diluted with pure isopropyl ether. In some cases the mixture was seeded with digitoxin or acetyldigitoxin that had been previously crystallized from the same solvent mixture. The flask was loosely stoppered and allowed to stand at room temperature or in the refrigerator until crystallization had taken place. The crystalline glycosides were collected and examined *per se*, after deacetylation and after hydrolysis by paper chromatography using solvent systems I and II. The hydrolysis of the glycosides was accomplished in the usual way. Deacetylation was effected by sodium hydroxide in the usual way and by the use of IRA-401 ion exchange resin. IRA-401 resin prepared in the usual way gave slow deacetylation in 85% aqueous methanol or ethanol. Complete and rapid (fifteen minutes) deacetylation was accomplished when the base form of the IRA-401 resin was used in a finely powdered form.

The results of the above studies showed that the crystalline glycosides were digitoxin and gitoxin from *Digitalis mertonensis* and *Digitalis purpurea*, and acetyldigitoxin from *Digitalis siberica*, *Digitalis ferruginea*, and *Digitalis amandiana*.

Figure 4 represents a typical chromatogram obtained in the characterization of these crystalline glycosides.

Figure 5 illustrates the use of system II for the

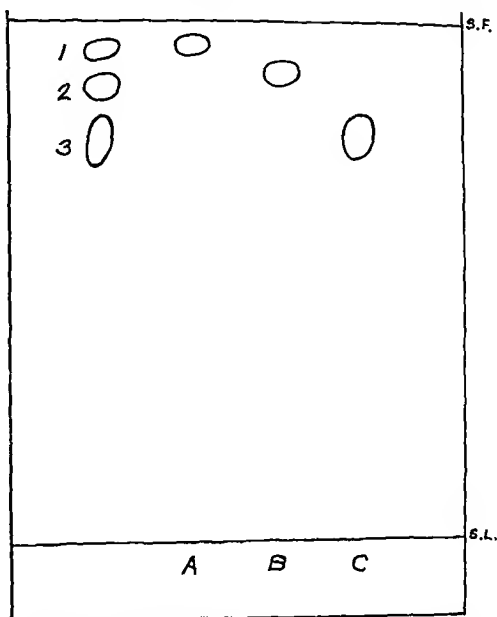


Fig. 4.—Solvent system I; S.F. = solvent front; S.L. = starting line; 1 = Acetyldigitoxin; 2 = digitoxigenin; 3 = digitoxin. A = Crystalline glycoside from *Digitalis siberica*; B = A hydrolyzed; C = A deacetylated. Raymond reagent used to locate the glycosides and aglyceous.

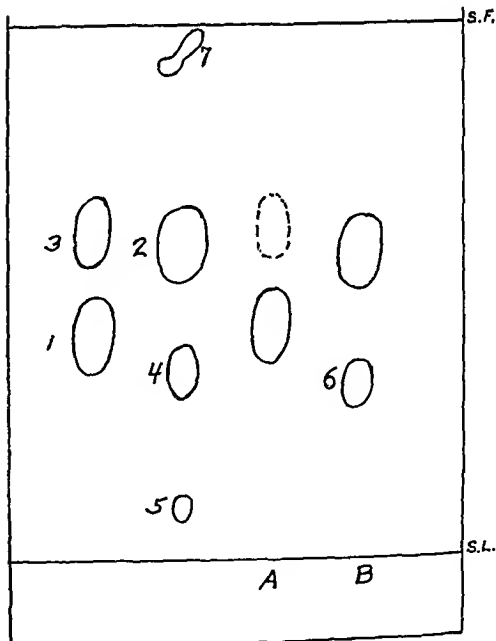


Fig. 5.—Solvent system II; S.F. = solvent front; S.L. = starting line. 1 = gitoxin; 2 = gitoxigenin from hydrolyzed gitoxin; 3 = impurity in gitoxin (gitoxigenin); 4, 5, and 6 = brown spots resulting from the hydrolysis of gitoxin and gitoxin from *Digitalis mertonensis*; 7 = anhydrogitoxigenin. A = gitoxin from *Digitalis mertonensis*; B = A hydrolyzed. Trichloroacetic acid reagent used to locate the glycosides and aglyceous.

characterization of the gitoxin that was obtained from *Digitalis mertonensis*. A blue spot was obtained for gitoxin, the gitoxin from *Digitalis mertonensis* and the first crop of crystalline material (See Fig. 3) from *Digitalis mertonensis* that has the same position on the paper as gitoxigenin. In some cases a bright blue spot followed the solvent front after the hydrolysis of the gitoxins. This possibly could be an anhydrogitoxigenin.

Figure 6 shows how excellent separation can be obtained between digitoxin and digitoxigenin at a temperature of 24° or slightly lower.

Digitoxin from *Digitalis Mertonensis*.—When extract A from 600 Gm. of fresh leaves of *Digitalis mertonensis* was concentrated to a small volume and allowed to evaporate slowly to dryness some of the glycosides crystallized. The mass thus obtained was carefully digested with methylisobutyl ketone or a mixture of methylene dichloride and ether (1:3). A clean separation of white crystalline glycosides was obtained that weighed 300 mg. The above results were reproducible. Digestion with methylene dichloride left a residue that was chiefly gitoxin. One recrystallization of the methylene dichloride soluble fraction from a mixture of methylene dichloride and ether gave 165 mg. of digitoxin that melted on the Koeffler Block at 221–223°. A second crop of 61 mg. of digitoxin was obtained.

The isolation of digitoxin from *Digitalis mertonensis* by the methods described in this paper appear to be both direct and simple. When this method was applied to *Digitalis purpurea* no direct separation of digitoxin was obtained from the initial extract A. Digitoxin was obtained in a crystalline state only when extract A was processed under the general procedure.

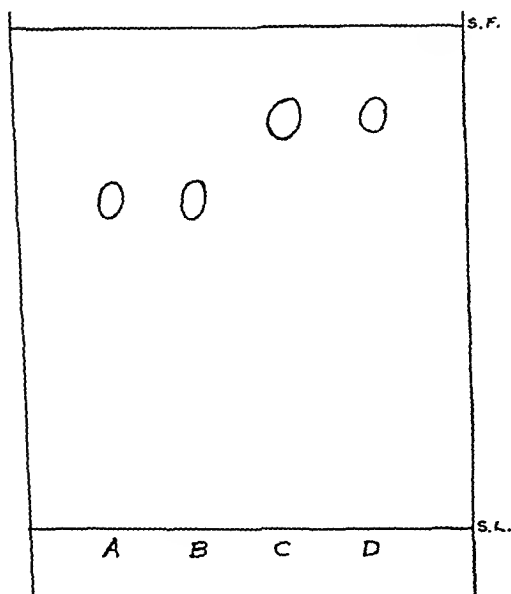


Fig. 6.—Solvent system I; S.F. = solvent front; S.L. = starting line. A = Digitoxin; B = crystalline glycoside from *Digitalis mertonensis*; C = A hydrolyzed; D = B hydrolyzed. Raymond reagent used to locate the glycosides and aglycons.

Enzyme Inhibiting Studies.—The following general procedure was adopted to examine the nature of the native digitalis glycosides found in the following digitalis species, i. e., *lanata*, *purpurea*, *ferruginea*, *mertonensis*, *amandiana*, and *siberica*. A small handful of leaves was placed in about 125 cc. of boiling distilled water and boiled for five minutes. In some cases the distilled water was buffered at pH 7 with phosphate.

A phosphate buffer was used to prevent any possible hydrolysis of the digitalis glycosides. Although no great differences between the use of distilled water and a phosphate buffer were observed, nevertheless, it appeared that better results were obtained when a phosphate buffer was used.

The mixture then was blended for five minutes and filtered. The filtrate was extracted as described under the general procedure and extracts A₁ and B₁ were obtained. These extracts were concentrated to dryness under a vacuum and then dissolved in about a 2-cc. mixture of equal parts of methylene dichloride and methanol. These extracts were examined paper chromatographically with the following results.

Figures 7 and 8 clearly demonstrate the absence of the desglucoglycosides. Extracts A₁ contain chiefly the A series of Stoll's native glycosides whereas extracts B₁ contain the C series and/or other glycoside substances. No attempt has been made at this time to resolve the minor glycosides found in the digitalis species investigated. Paper chromatograms clearly show that minor glycosides are present some of which may not be affected by the short

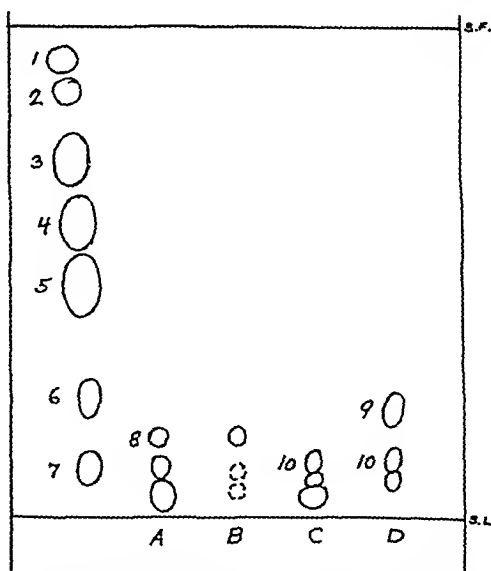


Fig. 7.—Solvent system II; S.F. = solvent front; S.L. = starting line. 1 = Acetyldigitoxin; 2 = digitoxin; 3 = acetyldigoxin; 4 = gitoxin; 5 = digoxin; 6 = lanatoside A; 7 = lanatoside C. A = extract B₁ from *Digitalis purpurea*; B = extract A₁ from *Digitalis purpurea*; C = extract B₁ from *Digitalis lanata*; D = extract A₁ from *Digitalis lanata*. Trichloroacetic acid reagent used to locate the glycosides.

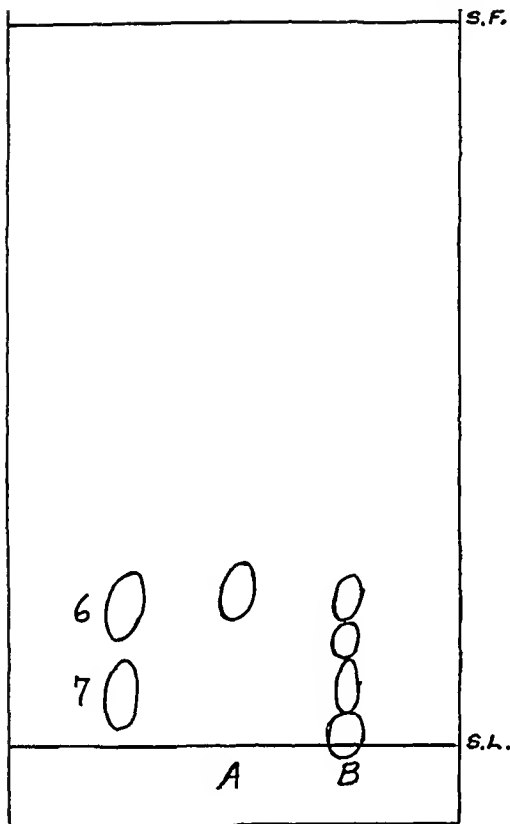


Fig. 8—Solvent system II 6 = Lanatoside A; 7 = lanatoside C A = extract A_1 from *Digitalis siberica*, B = extract B_1 from *Digitalis siberica* Trichloroacetic acid reagent used to locate the glycosides

period of enzymatic activity that hydrolyzes the glucose unit from the major glycosides This is probably the case with *digitalinum verum* found in *Digitalis lanata* and a glycoside found in *Digitalis purpurea* that moves on paper with solvent system II the same as *digitalinum verum*

Figure 7 shows the presence of purpurea glycoside A (No 8) in both extracts A_1 and B_1 , the remaining spots possibly could be purpurea glycoside B and other glycosides Lanatosides A and C are definitely shown to be present in extracts A_1 and B_1 as 9 and 10 respectively Other glycosides also are present

Figure 8 shows the presence of lanatoside A in extract A_1 Extract B_1 also contains some lanatoside A and what appears to be lanatoside C even though no acetyldigoxin was encountered in the extracts made from *Digitalis siberica* after a short fermentation period An intense deep blue spot remained at the starting line in extract B_1

In Fig 9, extract A_1 from *Digitalis mertonensis* shows the pronounced spots comparable to lanatoside A and B This was quite unexpected because no acetyldigoxin or acetylglitoxin could be detected in extracts that were made from this plant following a short fermentation period Lanatoside C appeared to be absent whereas the presence of lanatoside B is indicated A descending paper chro-

matogram was run for sixteen hours to verify the absence of lanatoside C and to confirm the presence of lanatoside B. Extract B_1 from *Digitalis mertonensis* gave a chromatographic picture the same as extract A_1 , however the spot for lanatoside A was weaker and the spot for lanatoside B was much brighter

The chromatographic results of extract A_1 from *Digitalis amandiana* was strikingly different from those obtained from the other species in that a very pronounced spot was obtained that indicated the presence of gitoxin in addition to one for lanatoside A. The presence of lanatoside A also was shown in extract B together with what appeared to be lanatoside B

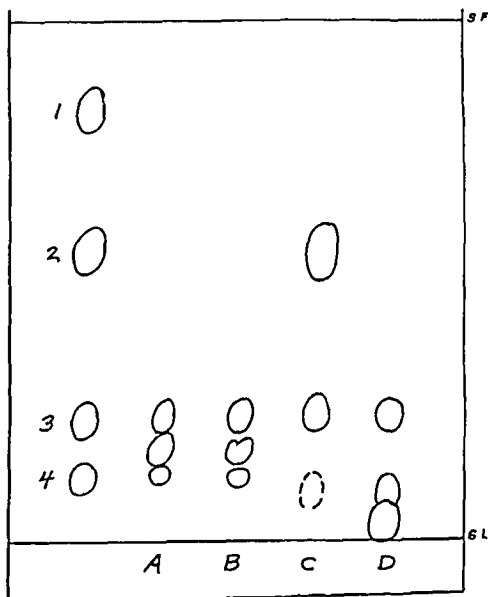


Fig. 9—Solvent system II; S F. = solvent front, S L = starting line 1 = Digitoxin; 2 = gitoxin; 3 = lanatoside A; 4 = lanatoside B A = extract A_1 from *Digitalis mertonensis*; B = extract B_1 from *Digitalis mertonensis*; C = extract A_1 from *Digitalis amandiana*; D = extract B_1 from *Digitalis amandiana* Trichloroacetic acid reagent used to locate the glycosides

Figure 10 is recorded to show the separation of lanatosides A, B, and C by the use of solvent system II by the descending technique after seven and one-half hours Shorter times do not separate lanatosides B and C, however longer periods than seven and one-half hours effect greater separations *Digitalinum verum* moves at a slower rate than lanatoside B and gives a bright blue-white fluorescence with the trichloroacetic acid reagent. Strosposide moves at the same rate as lanatoside A and gives a bright white fluorescence This chromatogram also shows the composition of some of the native glycosides of *Digitalis lanata* (enzyme inhibited extraction).

Chromatographic Analysis of Gitalin.—Gitalin when examined paper chromatographically (see

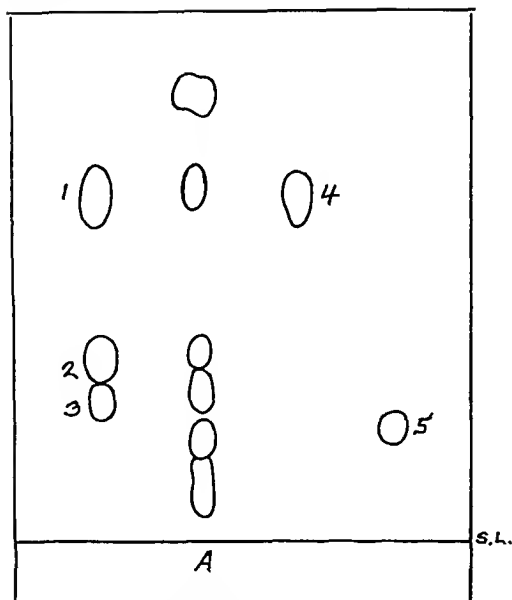


Fig 10—Solvent system II, descending technique, S L = starting line 1 = Lanatoside A, 2 = lanatoside B, 3 = lanatoside C, 4 = strosposide, 5 = *digitalinum verum*, A = extract prepared from enzyme inhibited *Digitalis lanata* Trichloroacetic acid used to locate the glycosides

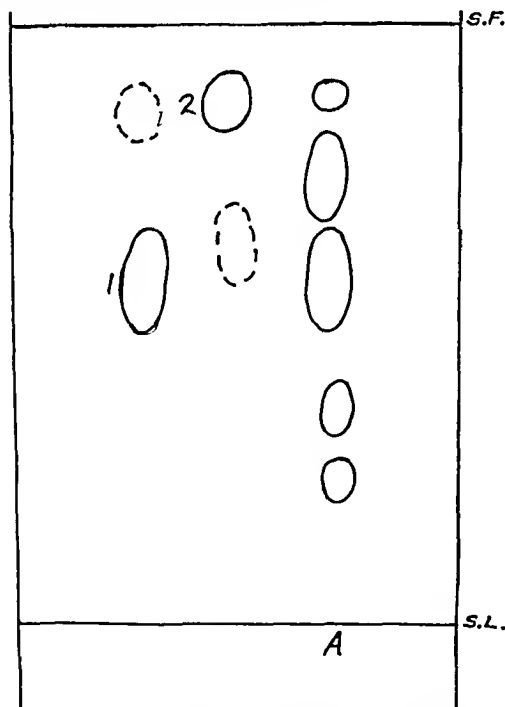


Fig 11—Solvent system II, S L = starting line, S F = solvent front 1 = Gitoxin, 2 = digitoxin, 3 = gitalin A = extract prepared from enzyme inhibited *Digitalis lanata* Trichloroacetic acid reagent used to locate the glycosides

Fig 11) using solvent system II showed the presence of five distinct spots one of which corresponded to gitoxin and another to digitoxin. The remaining spots gave a blue fluorescence under ultraviolet light.

Studies with Dried *Digitalis purpurea*.—Some fresh leaves of *Digitalis purpurea* were dried in an incubator at 60° overnight. The dried leaves were pulverized and extracts made as previously described. These extracts when examined by solvent system II definitely indicated that considerable digitoxin and gitoxin had been generated by enzymatic activity during the drying of the leaves, however, it was by no means complete.

The results of these preliminary studies are recorded in Fig 12.

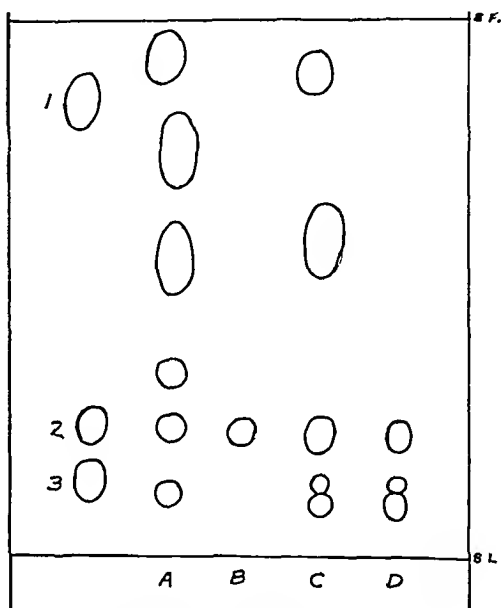


Fig 12—Solvent system II, S F = solvent front, S L = starting line 1 = digitoxin, 2 = lanatoside A, 3 = lanatoside C, A = extract A from *Digitalis purpurea* dry leaves, B = extract B from *Digitalis purpurea* dry leaves, C = extract A₁ from *Digitalis purpurea* dry leaves, D = extract B₁ from *Digitalis purpurea* dry leaves

REFERENCES

- (1) Schwartz S M, and Gisvold O. *THIS JOURNAL* 46, 324(1957), Appel R M and Gisvold O. *ibid* 43, 215 (1954) Hopponen R F and Gisvold O. *ibid* 41, 146(1952)
- (2) Stoll A and Kreis W, *Helv Chim Acta* 16, 120 (1934)
- (3) Jensen K B, and Tennoe K. *J Pharm and Pharmacol* 7, 334(1955)
- (4) Silberman H and Thorp R H. *ibid* 9, 401(1957)
- (5) Gisvold, O and Wright S I. *THIS JOURNAL* 46, 536(1957)
- (6) Schindler O and Reichstein T. *Helv Chim Acta* 34, 108(1951)
- (7) Svendsen A B and Jensen K B, *Pharm Acta Helv* 25, 241(1950) Jensen K B. *Acta Pharmacol toxicol* 9, 99(1953) Silberman H and Thorp R H. *J Pharm and Pharmacol* 6, 438(1953)

Enzymatic Decomposition of Digitalis Glycosides III*

By OLE GISVOLD

Enzyme inhibiting and enzyme favoring extraction techniques were applied to dried *Digitalis purpurea* leaves and to Digitalis Reference Standard. Paper chromatographic examination of the various extracts showed that digitalis leaves when dried carefully below 60° and Digitalis Reference Standard contained large amounts of the native glycosides such as purpurea glycoside-A. When ethanol or methanol concentrations of 66 per cent or greater were used to prepare the primary extracts enzymatic inhibition was obtained. A pH of one favored the enzymatic removal of the terminal glucose unit of the native glycosides.

PRELIMINARY phytochemical investigations from this laboratory have shown that when the fresh leaves of a number of digitalis species were extracted by a technique that permitted a short period of enzymatic activity, the desglucoglycosides could be isolated or detected by paper chromatographic techniques (1). If the fresh leaves were boiled for a short period of time before extraction, the native glycosides only could be detected by paper chromatographic methods. I had never intended to reinvestigate *Digitalis purpurea*, falsely believing that little remained to be done in this area. However, my experiences with the fresh leaves of the other species of digitalis led me to instigate some studies with dry leaves. The U. S. P. states that powdered digitalis is prepared from digitalis that has been dried at a temperature not exceeding 60°. I therefore dried some fresh leaves at 60° in an oven equipped with a blower. The leaves dried in a few hours and readily could be pulverized after twelve hours. When these leaves were examined by enzyme favoring and enzyme inhibiting techniques previously described (1) the results were somewhat comparable to those obtained from the fresh leaves. Some of the desglucoglycosides were present in the dried leaves and much of the native glycosides were also present. Dried leaves of *Digitalis purpurea* obtained from Callison Co. also were examined according to the same techniques and similar results were obtained. In this case it appeared that less desglucoglycosides were present in the dried leaf. An extract of the dried leaves made with 40 per cent (or less) methanol did not inhibit the enzyme that cleaved the terminal glucose residue from the native glycosides whereas 66 per cent (or stronger) methanol or ethanol completely inhibited the enzymatic activity.

When the fresh leaves of *Digitalis purpurea* were dried at 100° and subsequently examined by enzyme inhibiting techniques, only the desglucoglycosides could be detected. This

implied that enzymatic cleavage of the terminal glucose unit had taken place during the drying process. Because enzymatic activity is destroyed by plunging fresh or dried leaves into boiling water, it would appear that enzymatic activity must have been completed while the leaves were warming up to 100°. These studies being of a strictly qualitative nature were not able to detect any minute decompositions enzymatic or otherwise that may have taken place beyond the digitoxin stage.

An alcoholic (enzyme inhibiting concentrations) extract from powdered *Digitalis purpurea* containing the desglucoglycosides was prepared from a powdered leaf preparation that contained the native glycosides. The powdered leaf was first covered with a small amount of water and the mixture digested at 40 to 60° for about ten minutes to one hour. Alcohol was then added and the extraction carried out in the desired manner. Chromatographic examination of extracts prepared in this way revealed that most of the native glycosides had been converted to the desglucoglycosides.

After demonstrating that carefully dried leaves of *Digitalis purpurea* contained much native glycosides, it became necessary to determine the effect that a pH of one would have upon the enzymatic activity of the leaf because the whole powdered digitalis leaf is sometimes used orally. The powdered leaf was mixed with water having a pH 1 and allowed to stand with occasional shaking for one hour. After working up this preparation in the usual way I was able to show that the native glycosides had been converted chiefly to the desglucoglycosides. This demonstrated that the enzyme activity was not inhibited. The results also indicated that under the conditions of the experiment only removal of the terminal glucose residue had taken place.

Strospeside occurs in the extracts obtained from both the enzyme inhibiting and enzyme favoring procedures.

* Received April 25, 1958, from the College of Pharmacy, University of Minnesota.

Enzyme inhibiting and enzyme favoring techniques when applied to U. S. P. Digitalis Reference Standard 1942 also gave the same results that were obtained from the dried leaves. It can be concluded that this reference powder contained the active cardiac glycosides chiefly as the native glycosides. It also implied that the enzyme that cleaves the terminal glucose residue was still active.

The above results indicate that when U. S. P. Digitalis Reference Standard 1942 was and is used for biological assay purposes, the native glycosides are the chief reference glycosides. This is because the primary extract to be used for assay purposes is made with an alcohol concentration that completely inhibits the enzymes present. In the case of the digitalis leaf or powder to be assayed much would depend on where the leaf was grown and how it was dried and stored as to the nature and amounts of the glycosides.

There is a marked difference in the MLD $\mu\text{g./Kg.}$ in cats of purpurea glycoside-A which is 469 and digitoxin which is 330. The same is true for purpurea glycoside-B which is 548 and gitoxin which is 400. This is only part of the problem in the case of *Digitalis purpurea*. All prior biological assays of *Digitalis purpurea* are meaningless in terms of relating such values to oral activity in man. Although *Digitalis purpurea* may contain variable amounts (0 to very high percentages) of purpurea glycoside-B (or gitoxin) these glycosides are not absorbed orally in a cat and this also is very probably true in humans. Strospeside also occurs in variable amounts in *Digitalis purpurea* and even though it has a MLD $\mu\text{g./Kg.}$ in cats of 586, its physical properties probably preclude it from being absorbed orally. Nothing is known of the oral absorption of purpurea glycoside-A but it is probably considerably lower than digitoxin. Digitoxin on the other hand is claimed to be excellently absorbed if not 100 per cent.

It would appear therefore that an assay is needed to determine the oral potency of *Digitalis purpurea*. This might be done in animals if the results could be correlated in humans. An alternate solution would be an assay involving a determination of the amounts of the orally absorbable glycoside or glycosides by physical, chemical, or instrumental methods. In this case an assay for the digitoxin content probably would be most useful.

Prior assays (colorimetric) for the glycoside content of *Digitalis purpurea* also had little significance. This is because such assays were

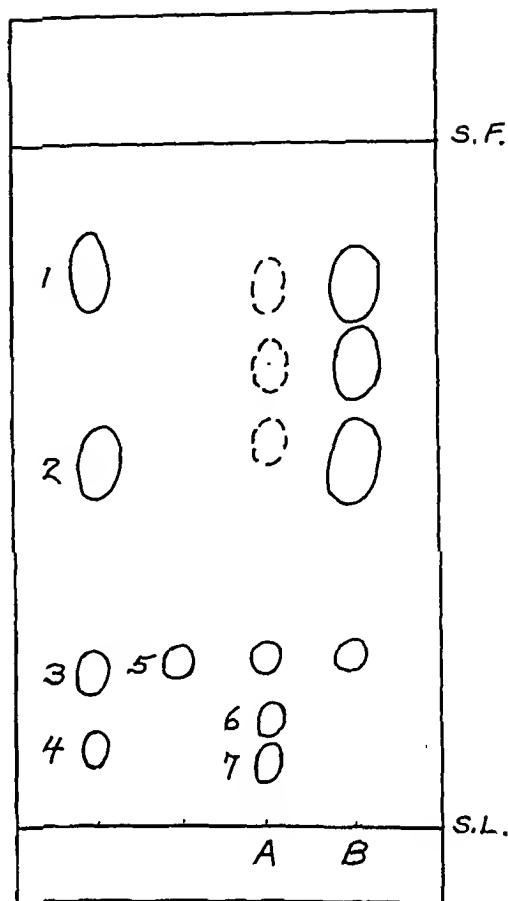


Fig. 1.—Solvent system II; S. F. = solvent front, S. L. = starting line. 1 = digitoxin; 2 = gitoxin; 3 = lanatoside-A; 4 = lanatoside-C; 5 = strospeside; 6 = purpurea glycoside-A; 7 = purpurea glycoside-B. A—Represents the chromatogram of an extract obtained from *Digitalis purpurea* powder dried at 60° or less or U. S. P. Digitalis Reference Standard. The extracts were prepared by the enzyme inhibiting techniques, i. e., boiling water or strong alcohol. B—Represents the chromatogram of an extract obtained from *Digitalis purpurea* powder dried at 60° or less or U. S. P. Digitalis Reference Standard. The extracts were prepared from the powdered leaf by a brief enzymatic treatment; one hour exposure to an aqueous medium at pH 1; alcohol concentrations of 40% or less or digestion with a small amount of water at 40 to 60° followed by ethanol. B—Also represents the chromatogram of an extract obtained by enzyme inhibiting techniques applied to digitalis leaf that had been dried at 100°.

based upon the presence of the α,β -unsaturated lactone ring which is present in all the cardio-active glycosides whether they are orally absorbed or not. Such assays suffered from the same disadvantages as did the biological assays.

EXPERIMENTAL

The details of the paper chromatographic techniques used in these studies have been described

previously (1). Solvent system II was used for the development of the paper chromatograms. Jensen's trichloroacetic acid reagent (2) was used to detect the position of the glycosides on the paper.

Extracts from Enzyme Inhibiting Treatment.—The following general procedure was used. Five grams of the powdered leaf was added to 100 cc. of boiling distilled water (or distilled water buffered to pH 7 with phosphate) and boiled for five minutes. The filtrate was then extracted with three successive portions of 30 cc. each of methylene dichloride. The methylene dichloride was removed and the residue dissolved in about two cc. of methanol for paper chromatographic examination.

When alcoholic concentrations (66% or higher) that inhibited enzymatic activity were used the drug (5 Gm.) was macerated overnight and the filtrate concentrated under vacuum to remove most of the alcohol. The concentrate was then diluted with water to make 100 cc. and then extracted three successive times with 25-cc. portions each of petroleum ether to remove much chlorophyll. The aqueous solution was then processed as above. If the final methanol solution was too highly pigmented it was partially depigmented by methods described in previous publications (1) or directly by treating it with small successive portions of the

finely powdered base form of the ion exchange resin IRA-401.

Extracts from Brief Enzymatic Treatment.—Five grams of the powdered leaf was added to 100 cc. of water and the mixture warmed to 50 to 60° for ten minutes. The filtrate then was treated as described under extracts from enzyme inhibiting treatment.

Extracts from 40% Methanol.—Five grams of powdered leaf was macerated overnight with 40% methanol and the filtrate processed as described where alcoholic concentrations of 66% or higher were used to make the initial extract.

Studies comparable to those described in the above experimental part have been initiated with *Digitalis mertonensis*. These will be completed and reported at a later date.

Figure 1 has been made by reconstructing a number of actual chromatograms in order to conserve space in THIS JOURNAL. The representations are so close to the actual chromatograms that little if any accuracy has been sacrificed.

REFERENCES

- (1) Gisvold, O., and Wright, S. F., THIS JOURNAL, 46, 535(1957); Gisvold, O., *ibid.*, in press.
- (2) Svendsen, A. B., and Jensen, K. B., *Pharm. Acta Helv.*, 25, 241(1950); Jensen, K. B., *Acta Pharmacol. Toxicol.*, 9, 99(1953); Silberman, H., and Thorp, R. H., *J. Pharm. and Pharmacol.*, 5, 438(1953).

A Study of the Oxidative Metabolism of Acetylsalicylic, Salicylic, and Gentisic Acid in Fevered Animals*

By ROSS E. CRABTREE,† JOHN B. DATA, and JOHN E. CHRISTIAN

Labeled compounds were used to study the metabolism of salicylates in rats. A method, electrophoretic separation, was developed to identify the oxidative metabolites. Analysis of tissue extracts by this method generally revealed only the administered compounds. However, gentisic acid was found in the pituitary region of fevered rats administered either acetylsalicylic or salicylic acid. It was not found in the pituitary after administration of these compounds to nonfevered rats or after administration of gentisic acid to fevered rats.

SEVERAL DRUGS form active metabolites, and in some cases these metabolites are actually responsible for the activity of the compounds. Salicylates are known to form at least one active metabolite, gentisic acid, which is found in the urine of subjects administered salicylates (1). This compound, 2,5-dihydroxybenzoic acid, has been shown (2, 3) to produce the same antipyretic effects as salicylates without the untoward

effects of "salicylism." However, authorities (1) state that it cannot be the active form of salicylate, because it must be given in the same high dosage as sodium salicylate in order to obtain the same therapeutic effects. It is also stated (4) that the amount formed in the body after the administration of sodium salicylate is not sufficient to exert the action.

Recent work (5, 6) has shown that there is selective accumulation of radioactivity in certain tissues of the body after administration of labeled salicylates. No work has been reported on the form in which the radioactive material is

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† Presented to the Pharmacy Section, AAAS, Indianapolis Meeting, December, 1957.

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present in these tissues. This study was undertaken to determine whether any metabolic products of salicylates are present in the tissues during the time in which the antipyretic action is being exerted.

EXPERIMENTAL

Synthesis of Possible Metabolites.—Gentisic acid was purchased commercially. It was electrophoretically pure and melted at 201–202° (corr.). 2-Acetylgentisic acid was prepared by the method of Bergmann and Dangsehat (7). It melted at 167–168° (corr.). It proved to contain gentisic acid as an impurity when examined by electrophoresis. 2,3,5-Trihydroxybenzoic acid was synthesized by the method of Schoek and Tabern (8, 9). It melted at 127–128° (corr.). It also proved to contain traces of gentisic acid when examined electrophoretically. Although gentisic acid was an impurity in both of these products, the compounds were sufficiently pure to establish their metabolic behavior.

Synthesis of Labeled Compounds.—C-14 Carboxyl salicylic and C-14 carboxyl acetylsalicylic acids were synthesized in these laboratories by Borst and Christian (10).

C-14 Carboxyl gentisic acid was synthesized from radioactive salicylic acid by the method of Brodie, *et al.* (11), using modifications necessary for the radioactive synthesis. To 250 ml. of 0.1 *M* phosphate solution, pH 6.6, there was added 207 mg. of C-14 salicylic acid, 87 mg. of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 474 mg. of ethylenediamine tetraacetic acid (EDTA), and 616 mg. of ascorbic acid. The solution was kept at 36° for four hours during which time air was vigorously bubbled into the solution. After acidification with hydrochloric acid the solution was extracted 4 times with 30 ml. of chloroform followed by 5 extractions with 30 ml. of ether. On evaporation the chloroform extract yielded 90 mg. of unreacted salicylic acid. The other extract contained the gentisic acid, crude yield 60%.

In order to prove the identity of the compound isolated, a portion of the product was subjected to separation on a chromatographic column according to the general method described by Trenner (12). The solvent pair was glycerol-ethyl acetate and the holding solid was Supercel. The progress of the material on the column was followed by its fluorescence under ultraviolet light. The fluorescent material was eluted from the column, and after evaporation of the solvent, white crystals remained. The product was electrophoretically pure, radiochemically pure, and it had the same melting point as gentisic acid obtained commercially. The specific activity determined in the Aetigraph apparatus¹ was 740 c. p. m./mg.

Procedure.—Fever was produced in the animals by use of peptone solution according to the procedure given by Buller, *et al.* (13). The drugs were dissolved in 1 ml. of 0.1 *M* phosphate solution, pH 7.4, and administered by subcutaneous injection. Table 1 shows the dosage schedule in which the various compounds were administered. Forty-five minutes after injection the rats were decapitated,

TABLE I.—DOSAGE SCHEDULE FOR SALICYLATE COMPOUNDS ADMINISTERED TO RATS

Compounds	Dose, mg./Kg.	No. of Rats in Group	Condition
C-14 Acetylsalicylic acid	100	5	Febrile
Acetylsalicylic acid	100	10 ^a	Febrile
C-14 Acetylsalicylic acid	100	2	Nonfebrile
Acetylsalicylic acid	100	10 ^a	Nonfebrile
C-14 Salicylic acid	200	5	Febrile
C-14 Gentisic acid	200	6	Febrile
2-Acetylgentisic acid	150	3	Febrile
Gentisic and 2,3,5-trihydroxybenzoic acid mixture	100	3	Febrile

^a Only the blood, urine, and pituitary were examined in these experiments.

and the blood and urine collected. The tissues were dissected, frozen on dry ice, and stored until use.

The blood was collected over sodium citrate and immediately centrifuged. The plasma and red cells were then separated and an equal volume of acetone added to each of these fractions. Each of the plasma samples was treated separately, but the red cell extracts were pooled for measurement. The mixtures were centrifuged and the supernatant liquids dried over sulfuric acid in a vacuum desiccator at 36°.

The rest of the tissue samples were also pooled. The liver, kidneys, heart, spleen, lungs, adrenals, brain, and pituitary² were all separately homogenized with alcohol, centrifuged, and the supernatants evaporated *in vacuo* at 36°.

Electrophoresis.—All the compounds were subjected to electrophoresis on the Reeco apparatus.³ The separations were made on Schleicher and Schuell 2045a filter paper strips. Citrate buffer was used, pH 4.0, ionic strength 0.15. The compounds were dissolved in 1:1 ethanol/buffer solution and 0.02 ml. was applied to the negative side of the strips. Electrophoresis was run for five hours at 350 v. which gave a current of approximately 10 ma.

The spots were detected on the strips by examination under ultraviolet light, by reaction with ferric nitrate solution, and by radioactivity. The strips were scanned for radioactivity by the use of the Aetigraph apparatus. The slits furnished with the instrument were replaced with a lead disk containing a 0.5-cm. slit which was positioned 1 mm. above the paper strips.

RESULTS

Various salicylate derivatives can be distinctly separated by electrophoresis. Table II gives the relative movement of the various compounds. Salicylic acid was arbitrarily assigned a *R_f* value of 1.0 and the movement of the compounds compared to it.

¹ In the case of the pituitary a section of the surrounding brain tissue on the floor of the third ventricle was also included.

² Model No. E-800-2, Research Equipment Corp., Oakland Calif.

³ Model No. C-100-Nuclear Instrument and Chemical Corp., Chicago, Ill., 1.4 mg./cm.² mica window.

TABLE II.—ELECTROPHORETIC SEPARATION OF SALICYLATE DERIVATIVES

Compounds	R _f Value
Salicylic acid	1.00
Gentisic acid	0.80
Acetylsalicylic acid	0.70
2-Acetylgentisic acid	0.62
2,3,5-Trihydroxybenzoic acid	0.58

Acetylsalicylic Acid.—Table III shows the compounds detected in the various tissues of the fevered rats administered C-14 acetylsalicylic acid. A rough estimate of the relative concentrations of the compounds was made by comparison of the areas under the radioactivity curves. In most cases the curves overlapped so that definite quantitative values could not be assigned.

to two nonfevered rats. The findings were generally the same as those with fevered rats except that gentisic acid was not found in the pituitary extracts. Confirmatory experiments with nonradioactive acetylsalicylic acid also failed to show any fluorescent spot in the pituitary strips for nonfevered rats.

Salicylic Acid.—The results of the administration of C-14 salicylic acid were limited by the low specific activity of the compound. Only qualitative results were recorded. Salicylic acid was the only radioactive compound found in the plasma, red blood cells, heart, lungs, spleen, liver, and brain. Gentisic acid was again detected in the kidneys, urine, and pituitary extracts. An unidentified compound was found in the adrenal extract with the same *R_f* value, 0.15, as found in the previous experiments.

TABLE III.—COMPOUNDS FOUND IN THE TISSUES AFTER ADMINISTRATION OF C-14 CARBOXYL ACETYL SALICYLIC ACID TO FEVERED RATS

Tissue	Acetylsalicylic Acid	Salicylic Acid	Gentisic Acid	Unknown (R _f Value)
Plasma	++++ ^a	+
Red blood cells	++	++
Kidneys	..	+++	+	...
Urine	..	++++	++	+(0.19) ++(0.57)
Heart	+	+++
Lungs	+	+
Spleen	+	++
Liver	++	+++
Brain	+	+
Pituitary	+	+	+	...
Adrenals	+	+	..	+(0.15)

^a The concentrations are graded from + to ++++ according to the relative areas under the radioactivity curves.

In general, only acetylsalicylic and salicylic acids were found in the tissues. However, certain exceptions are noteworthy. Gentisic acid was found in the kidneys, urine, and pituitary extracts. Two unidentified products were detected in the urine. One unidentified product was also found in the adrenal extract.

The three radioactive spots on the pituitary strip were sufficiently separated so that they could be cut out and the radioactivity determined under a Geiger-Müller tube. The three sections corresponding to salicylic, gentisic, and acetylsalicylic acids gave equal activity demonstrating that gentisic acid was present in the pituitary in approximately equal concentration to the amount of salicylic acid and to the amount of acetylsalicylic acid. The section corresponding to gentisic acid was eluted with alcohol and pure gentisic acid was added. Electrophoresis of this solution gave only one spot. The spot was fluorescent and contained all the radioactivity. This was taken as proof that the compound in the pituitary was gentisic acid.

For confirmation of these findings two groups of five fevered rats were administered acetylsalicylic acid. In both groups the fluorescent spot corresponding to gentisic acid appeared on the pituitary strips. Gentisic acid was also found in the urine but not in the plasma.

C-14 Acetylsalicylic acid was also administered

Gentisic Acid.—The labeled gentisic acid was also of low specific activity and only qualitative results were recorded. Gentisic acid was found in most of the tissue examined. A radioactive peak corresponded to the bright blue fluorescent spot of gentisic acid in every case except the pituitary. In the pituitary a radioactive spot, *R_f* value 0.73,⁴ was found, but no fluorescence was observed. In a number of other tissues radioactivity was also recorded in a nonfluorescent section with a *R_f* value of approximately 0.70. These tissues were the plasma, red cells, liver, and adrenals. In the urine two fluorescent spots were observed to be radioactive, *R_f* values of 2.01 and 1.21. There was a third spot in the urine which did not fluoresce but was radioactive, *R_f* value of 0.63.

Apparently gentisic acid occurs in widespread body distribution. The widespread occurrence of the nonfluorescent compound indicates that gentisic acid is changed by the body into another form which does not possess fluorescent properties.

2-Acetylgentisic Acid.—Only one fluorescent spot corresponding to gentisic acid could be detected on the electropherograms after the administration of 2-acetylgentisic acid to fevered rats. Hydrolysis of the spots by placing the strips in an ammonia

⁴ In these results gentisic acid was used as the comparison compound (*R_f* = 1.0).

atmosphere failed to reveal a second spot for 2-acetylgentisic acid. The pituitary again had no fluorescence to indicate the presence of gentisic acid. The urine patterns corresponded to those found after the administration of gentisic acid. Apparently 2-acetylgentisic acid is quickly hydrolyzed in the body.

2,3,5-Trihydroxybenzoic Acid.—Administration of the mixture of gentisic and 2,3,5-trihydroxybenzoic acids gave electrophoretic patterns which were quite similar to those of gentisic acid itself. In most of the tissues only the two administered compounds were detected. However, the plasma and red cells produced only one spot, that of gentisic acid. The trihydroxy acid could not be recovered from plasma when added *in vitro* which may explain why it could not be detected *in vivo*.

The urine strips showed a definite spot for the trihydroxy acid. This indicates that the trihydroxy compound is at least partly excreted unchanged.

DISCUSSION

Pallot and Eberhardt (6) have reported that after the administration of labeled sodium salicylate to rats the radioactivity accumulates in the anterior pituitary in a greater concentration than in any other tissue except the blood. In the present work it was shown that in febrile rats a significant part of this radioactivity in the pituitary is composed of gentisic acid. The presence of this metabolite in the pituitary is highly significant since many workers believe that one of the main actions of salicylates is activation of the hypothalamic-hypophyseal-adrenal axis (14-16). Since a high concentration of gentisic acid was found in this region, it was actually present at the site of action in sufficient quantity to exert the action. The absence of gentisic acid in the pituitary of non-febrile rats might be one reason for the failure of salicylate to lower normal body temperature to subnormal levels.

Udenfriend, *et al* (17), found that a mixture of ascorbic acid, EDTA, and ferrous ion will bring about hydroxylation of a number of drugs. In this connection it may be pointed out that ascorbic acid occurs in high concentration in the pituitary (18). This fact, combined with the relatively high concentration of radioactive salicylate which accumulates there, indicates that this could be one mechanism by which the gentisic acid is formed in the pituitary.

The effects which occur after the administration of acetylsalicylic acid are complex and cannot be explained solely by activation of the hypothalamic-hypophyseal-adrenal axis. The total effect arises from stimulation or depression of several systems of the body. Since widespread body distribution of any salicylate metabolite was not found, the peripheral actions of salicylate are probably due to the salicylate itself. This does not exclude the possibility that the salicylate may form complexes with certain compounds of the body which in turn may exert these actions.

Dumazert and El Ouachi (19) detected levulinic acid in the urine of rabbits and humans after the administration of sodium salicylate. They postu-

lated that it arose from rupture of the ring of 2,3,5-trihydroxybenzoic acid during these hydrolysis procedure. In the past work it was shown that 2,3,5-trihydroxybenzoic acid is partly excreted unchanged. The trihydroxy acid spot was identified by its yellow color in visible light and its strong fluorescence under ultraviolet. 2,3,5-Trihydroxybenzoic acid probably is not a metabolite of salicylates in rats. A spot with approximately the same R_f value was also found in the urine of rats treated with acetylsalicylic acid, but the spot was not yellow in visible light and did not fluoresce under ultraviolet. Hence, the spot was not 2,3,5-trihydroxybenzoic acid. The unidentified spots found on the urine strips were probably conjugated products since hydrolysis of the urine strips in an ammonia atmosphere and separation of the alcoholic eluates gave only spots for gentisic and salicylic acids.

SUMMARY

1. A method of electrophoretic separation was developed for oxidative metabolites of salicylates.

2. Synthesis of possible salicylate metabolites is reported. These are C-14 carboxyl gentisic acid, 2-acetylgentisic acid, and 2,3,5-trihydroxybenzoic acid.

3. The metabolic products found in various rat tissues after the administration of labeled acetylsalicylic acid, salicylic acid, and gentisic acid were determined. Gentisic acid was found in the pituitary, kidneys, and urine after administration of acetylsalicylic acid and salicylic acid to febrile rats but not in any other tissues.

REFERENCES

- (1) Goodman, L. S., and Gilman, A., "The Pharmacological Basis of Therapeutics," 2nd ed., Macmillan Co., New York, 1955, p. 299.
- (2) Clarke, N. E., and Mosher, R. E., *Circulation*, **7**, 337(1953).
- (3) Schaefer, L. E., Rashkoff, I. A., and Megibone, R. S., *ibid.*, **2**, 265(1950).
- (4) Batterman, R. C., and Sommer, E. M., *Proc. Soc. Exptl. Biol. Med.*, **82**, 376(1953).
- (5) Borst, W. R., Miya, T. S., and Christian, J. E., *THIS JOURNAL*, **45**, 511(1956).
- (6) Pallot, G., and Eberhardt, H., *Compt. rend. soc. biol.*, **150**, 1220(1956).
- (7) Bergmann, M., and Dangschat, P., *Ber.*, **52B**, 371(1919).
- (8) Schock, R. U., Jr., and Tabern, D. L., *J. Org. Chem.*, **16**, 1772(1951).
- (9) Schock, R. U., Jr., and Tabern, D. L., *U. S. Pat.* 2,641,609, June 9, 1953.
- (10) Borst, W. R., and Christian, J. E., *THIS JOURNAL*, **45**, 23(1956).
- (11) Brodie, B. B., Axelrod, J., Parkhurst, A. S., and Udenfriend, S., *J. Biol. Chem.*, **208**, 741(1954).
- (12) Trenner, N. R., "Column Partition Chromatography," Merck and Co., Inc., Rahway, N. J., 1957.
- (13) Butler, R. H., Miya, T. S., and Carr, C. J., *J. Pharm. and Pharmacol.*, **9**, 128(1957).
- (14) Eades, C. H., Jr., and King, J. S., Jr., *Intern. Record Med.*, **169**, 642(1956).
- (15) Applezweig, N., *Drug & Cosmetic Ind.*, **75**, 457(1954).
- (16) George, R., and Way, E. L., *J. Pharmacol. Exptl. Therap.*, **119**, 310(1957).
- (17) Udenfriend, S., Clark, C. T., Axelrod, J., and Brodie, B. B., *J. Biol. Chem.*, **208**, 731(1954).
- (18) King, C. G., "The Vitamins: A Symposium," American Medical Association, Chicago, 1959, p. 333.
- (19) Dumazert, C., and El Ouachi, M., *Ann. pharm. franc.*, **12**, 723(1954).

The Relationships between the Histamine Liberating and Hypotensive Actions of 1-Phenyl-2-Dimethylaminoethoxymethyl Tetrazole Hydrochloride (TT-209)*

By M. B. SLOMKA

1-Phenyl-2-dimethylaminoethoxymethyl tetrazole hydrochloride (TT-209) produces a profound hypotension of long duration in the dog; refractoriness to the hypotensive action of TT-209 develops on repeated administration. TT-209's ability to produce hypotension is associated with its ability to increase plasma histamine concentrations. TT-209 and compound 48/80 demonstrate a cross-refractoriness in their ability to affect dog blood pressure and plasma histamine concentration. It is concluded that the hypotensive action of TT-209 is mediated via a mechanism of histamine liberation.

FROM AN EXTENSIVE ANALYSIS of the hypotensive action of 1-phenyl-2-dimethylaminoethoxymethyl tetrazole hydrochloride (TT-209), Yim, Gross, and Keasling suggested that this action of the compound was due to its direct depressant action on the cardiac and vascular smooth muscle (1). The similarity of the hypotensive action of TT-209 to that of known histamine liberators suggested that this action of the compound might be mediated via a mechanism of histamine liberation. This possibility was tested in the experiments reported in this communication.

EXPERIMENTAL

Methods.—Blood pressure was recorded by means of a mercury manometer from a common carotid artery of mongrel dogs anesthetized with sodium pentobarbital 33 mg./Kg. All drugs were injected rapidly as saline solutions into a femoral vein. In those experiments in which blood samples were obtained for analysis of plasma histamine the samples were withdrawn from the contralateral femoral vein from one to two minutes after drug injection into heparinized syringes.

Histamine bioassay was performed on guinea pig ileum strips suspended in an atropinized (atropine sulfate 10^{-6}) Tyrode's solution at 37° . Strips were standardized to histamine prior to each addition of dog plasma. Pharmacologic identification of histamine in plasma was established by the ability of Benadryl (10^{-7}) to inhibit plasma contractions to the same degree as histamine contractions.

Results.—The prolonged hypotensive effect of 10 mg./Kg. TT-209 in pentobarbital anesthetized dogs and the development of refractoriness to this action of the compound is illustrated in Fig. 1. The TT-209 refractory dog demonstrates a cross-

refractoriness to the hypotensive action of 200 μ g./Kg. compound 48/80 but not to the hypotensive action of 0.1 cc./Kg. 10% Tween 20.

The administration of 200 μ g./Kg. compound 48/80 produces a hypotension similar in intensity and duration to that seen with TT-209; the animals are refractory to further administration of compound 48/80. The compound 48/80 refractory animal demonstrates a cross-refractoriness to the hypotensive action of 10 mg./Kg. TT-209.

The mean fall in blood pressure of 86% produced by 10 mg./Kg. TT-209 was associated with a mean increase in plasma histamine concentration of 0.35 μ g./cc. (Table I); 200 μ g./Kg. compound 48/80 produced a similar mean fall in blood pressure, 85%, and mean increase in plasma histamine concentration, 0.44 μ g./cc., (Table III). In dogs made refractory to TT-209 or compound 48/80 by prior administration of either compound TT-209 or compound 48/80 produced at most transient depressor effects that were associated with no measurable increases in plasma histamine concentration. (Tables II and III).

Discussion.—The magnitude and duration of the hypotensive response and the development of tolerance to 10 mg./Kg. TT-209 agrees with that previously reported by Yim, Gross, and Keasling (1). The hypotensive activity and the development of refractoriness to such activity of compound 48/80 has been previously reported (2, 3, 4). Evidence

TABLE I.—THE EFFECT OF 10 MG./KG. TT-209 ON DOG BLOOD PRESSURE AND PLASMA HISTAMINE CONCENTRATION

Dog No.	Blood Pressure Control, mm. Hg	Blood Pressure Fall, %	Histamine Plasma Control	Histamine Plasma Concn., μ g./ml 1-2 Min. Post Drug
B	160	88	None ^a	0.21
E	124	81	None ^a	0.36
F	122	85	None ^a	0.36
I	144	83	None ^a	0.23
GG	128	87	None ^a	0.36
JJ	138	90	None ^a	0.30
KK	110	92	None ^a	0.32
QQ	136	90	None ^a	0.59
$\bar{x} \pm S_x$		86 ± 1.3		0.35 ± 0.01

^a None indicates less than 0.063 μ g./0.5 ml., the plasma volume used to assay for histamine.

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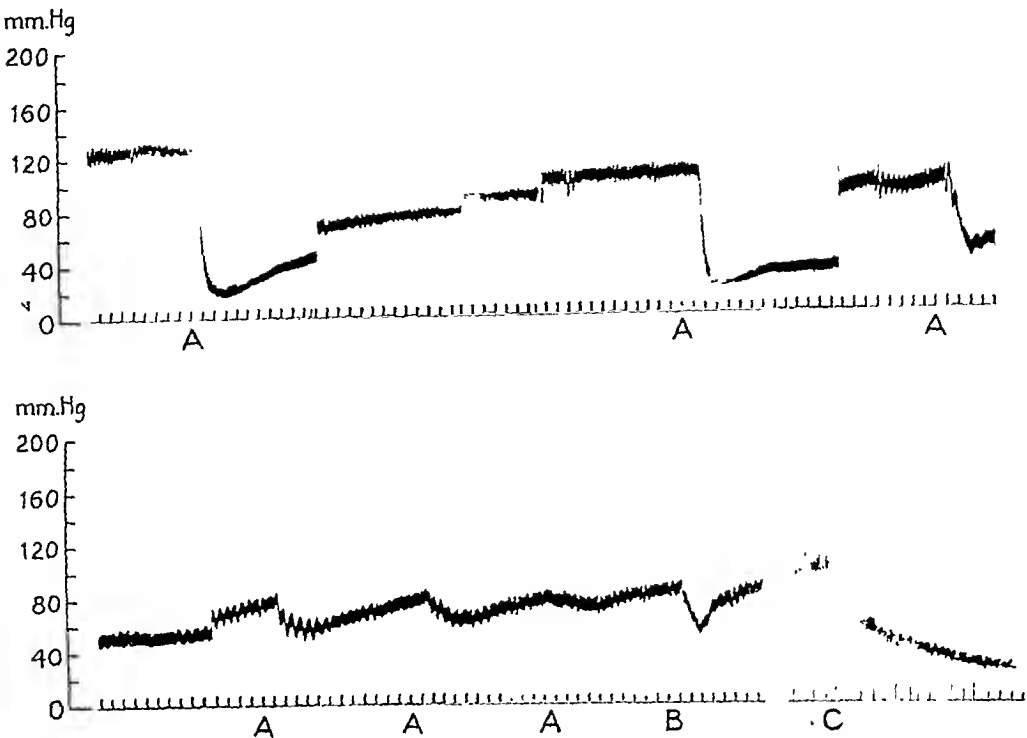


Fig. 1.—Carotid blood pressure, time marks one minute; kymograph stops of fifteen to thirty minutes. Legend:—A marks injection of 10 mg./Kg. TT-209, B marks injection of 200 μ g./Kg. compound 48/80; C marks injection of 0.1 ml./Kg. 10% Tween 20.

TABLE II.—THE EFFECT OF 10 MG./KG. TT-209 ON DOG BLOOD PRESSURE AND PLASMA HISTAMINE CONCENTRATION AFTER PRETREATMENT

Pretreatment Dog No.	Blood Pressure Control mm. Hg	Fall, %	Histamine Plasma Concn., μ g./ml. Control	1-2 Min. Post Drug
Refractory to 10 mg./Kg. TT-209				
E	118	17	None ^a	None ^a
F	78	0	None ^a	None ^a
GG	82	24	None ^a	None ^a
LL	104	10	None ^a	None ^a
NN	144	87	None ^a	None ^a
Refractory to 200 μ g./Kg. compound 48/80				
H	140	17	None ^a	None ^a
J	106	5	None ^a	None ^a
OO	100	0	None ^a	None ^a
$\bar{x} \pm S_x$		20 \pm 10		

^a None indicates less than 0.063 μ g./0.5 ml., the plasma volume used to assay for histamine.

indicates liberation of endogenous histamine to be the mechanism by which compound 48/80 exerts its hypotensive activity (2-5). The cross-refractoriness shown between TT-209 and compound 48/80 in their ability to produce their prolonged hypotensive responses would indicate that TT-209 is also producing this effect via a mechanism of endogenous histamine liberation. This mechanism of action is supported by the apparent relationships between the ability of TT-209 to produce its characteristic effect on the blood pressure and to affect plasma histamine concentration in previously untreated dogs and its

inability to affect these parameters in TT-209 or compound 48/80 refractory dogs. The inability of diphenhydramine to block the hypotensive response to TT-209 (1) is not inconsistent with the proposed hypothesis. Although antihistaminic agents can block the response to small doses of histamine, they are not effective in blocking the hypotensive response to large doses of exogenous histamine (6) or the amounts of histamine presumably released in close proximity to histaminergic receptors by histamine liberators or in anaphylaxis (7).

TABLE III —THE EFFECT OF 200 µG /KG COMPOUND 48/80 ON DOG BLOOD PRESSURE AND PLASMA HISTAMINE CONCENTRATION

Pretreatment Dog No	Blood Pressure		Histamine Plasma Concn , µg /ml Control	1-2 Min Post Drug
	Control, mm Hg	Fall, %		
None	110-160	85	0 13 ± 0 04	0 57 ± 0 10
	110-160	85	None ^a	0 44 ± 0 05
Refractory to 200 µg /Kg compound 48/80	80-160	0-10	None	None
Refractory to 10 mg /Kg TT-209				
GG	78	59	None	None
LL	108	0	None	None
NN	146	0	None	None
TT	119	30	None	None
$\bar{x} \pm s_x$		22 ± 14		

^a None indicates less than 0 063 µg /0 5 ml , the plasma volume used to assay for histamine

The ability of another known histamine liberator, Tween 20 (8), to produce prolonged hypotension in the TT-209 compound 48/80 refractory dog indicates (a) a hypotensive response can still be attained in the animal, (b) the animal's releasable histamine stores have not been depleted, and (c) TT-209 in the dosage employed does not possess a significant degree of antihistaminic activity on the dog's vascular system

SUMMARY

- 1 Ten mg /Kg. TT-209 produces prolonged hypotension in the dog that is associated with an increase in plasma histamine concentration; refractoriness develops with repeated administration.
2. TT-209 and compound 48/80 demonstrate

cross-refractoriness with regard to their ability to affect the blood pressure and plasma histamine concentration.

3. Evidence indicates that TT-209 produces its hypotensive action via a mechanism of histamine liberation.

REFERENCES

- (1) Yim, G K W, Gross, F G , and Keasling, H , THIS JOURNAL, 45, 393(1956)
- (2) Eaton, W D M , *Brit J Pharmacol* , 6, 400(1951)
- (3) Dews, P B , et al , *J Pharmacol and Exptl Therap* , 107, 1(1953)
- (4) Slomka, M B , and Goth, A , *Proc Soc Exptl Biol Med* , 93, 30(1956)
- (5) Feldberg, W , and Talesnik, J , *J Physiol* , 120, 550(1953)
- (6) Wells, J A , et al , *J Pharmacol and Exptl Therap* , 85, 122(1945)
- (7) Dale, H H , *Brit Med J* , 11, 281(1948)
- (8) Krantz, J C , et al , *J Pharmacol and Exptl Therap* 93, 188(1947)

Zein as a Film-Type Coating for Medicinal Tablets^{*}

By EDWARD P. WINTERS and DWIGHT L. DEARDORFF†

Zein, with and without certain additives, was added to isopropyl alcohol. Tablets coated with the resultant nonenteric fluids adequately withstand heat, abrasion, and humidity. Tastes and odors of the original tablets are concealed by the coating. Grooves and emblems on the tablet surface are retained and remain visible through the coating. The differences in solubility between the coated and uncoated tablets are negligible.

UTILIZING ESTABLISHED METHODS of sugar coating, one of the larger pharmaceutical manufacturers in this country employs more than 150 coats to cover one of their tablet products adequately. This entire process, based on a regular working schedule, requires as many as five working days. While the resultant coated

tablets are pharmaceutically elegant, much remains to be accomplished with respect to decreasing the time and labor involved.

Perhaps the most expedient method of accomplishing this objective would be the development of a coating which would serve the general purposes of a nonenteric coating (1-3) without the use of subcoat powders and buildup coatings; that is, a "film-type" coating. At least one pharmaceutical manufacturer is currently producing tablets coated in this manner. Two of the more recent endeavors along these lines have been per-

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formed by Gans and Chavkin (4) and Doerr, *et al.* (5). An effort has been made to produce a nonenteric coating fluid using zein which would not require the numerous coats now employed, thereby resulting in a substantial diminution in equipment needs. Zein is resistant to microbial attack (6) and is considered to be nontoxic and suitable for use in functional coatings involving the ingestion of relatively small amounts of material (7).

EXPERIMENTAL

Materials.—The following abbreviations are used for zein and nonionic surfactants employed in this study: (Z) Zein, (T20) polyoxyethylene sorbitan monolaurate, (T80) polyoxyethylene sorbitan monooleate, (S20) sorbitan monolaurate, (S80) sorbitan monooleate, (A83) sorbitan sesquileate, (Ax) aspirin tablets, 340 mg., Manufacturing pharmacy Laboratories, University of Illinois, (Pa) placebo tablets, 250 mg., Abbott Laboratories, 621-8914, as described by Doerr, *et al.* (5), from basic starch-lactose granulation, (Px) placebo tablets, 350 mg., Manufacturing Pharmacy Laboratories, University of Illinois, from basic starch-lactose granulation as used by Koren (8) and Villacorta (9).

Tablets (Commercially Coated).—Ammonium chloride, 7½ gr., enteric coated, Parke Davis and Company: cascara sagrada extract, 5 gr., Upjohn Company: ferrous gluconate, 5 gr., "Fergon," Winthrop Laboratories: ferrous sulfate, 3 gr., "Feosol," Smith Kline and French Laboratories: sodium salicylate, 10 gr., enteric coated, Abbott Laboratories.

Apparatus.—The apparatus used in this study included the following: 20-inch galvanized iron coating pan, 20-inch galvanized iron canvas lined polishing pan—both the coating and polishing pans were interchangeable on a motor driven bench stand and revolved at a speed of 36 r. p. m. Industrial vacuum machine (General Electric Model 203), Fisher Twin Infra-Red Radiator (each bulb rated 250 watts), U. S. P. Disintegration Device (Scientific Glass Apparatus Company, Inc.), Shaking machine (A. S. Aloe Company), Glass Desiccator Jar, 250 mm. (Central Scientific Company), and 5X Aplanat Magnifying Lens (Bausch and Lomb).

Methods.—The coating formulas used may be explained as follows: the figures immediately following the abbreviations previously described represent the percentage by weight of the preceding material. In every instance the remainder of the formula is understood as being 91% (v/v) isopropyl alcohol, calculated on a weight basis according to the prescribed formula. For example, the formula Z-15-(T20)-3 should be read as 15% zein, 3% polyoxyethylene sorbitan monolaurate, and 82% isopropyl alcohol (91% by volume): the percentages in the formula all being on a w/w basis.

Application of Zein-Additive Fluids.—The tablets were screened, weighed, and placed in the pan ready for coating. The first application of coating fluid was added almost immediately to lessen the possibility of the uncoated tablets clumping while tumbling in the rotating pan. The fluid was applied in a thin stream along the top of the tumbling tablets.

The tablets and coating were not warmed prior to application.

A maximum interval of three minutes is required between applications of the coating fluid, providing that warm air is directed onto the tablets. This time interval is dependent upon the amount of time required for the previous coating to dry. A warm air blast is necessary to shorten the drying period of each coating, thereby preventing the tablets from adhering to one another or to the sides of the pan. It is of utmost importance that this warm air (54°) be applied only after the coating has been completely distributed over all the tablets. If this procedure is not adhered to closely, the coating fluid will dry before it has covered the entire batch, resulting in an uneven coating.

When every visible tablet is evenly covered with a very thin coating of the fluid, the warm air is applied immediately. An excessive quantity of fluid produces a rough, uneven coating and leads to the possibility of tablets clumping together. The warm air treatment will usually relieve this situation, but generally when the tablets separate they are irreparably marked at the point of previous contact. Shellac sealcoat and wax polishing solutions were prepared by standard methods employing the general formulas as commonly used in industry (see Table I).

Addition of Colors.—Varying concentrations of FD & C Blue No. 1 (Brilliant Blue) and FD & C Red No. 3 (erythrosine) in Z-15-(T20)-3 were prepared. The majority of the coloring experiments were performed using the red dye since this was decided upon as being the more popular and appealing of the two colors employed.

Concentrations ranging from 0.02% to 0.5% (w/w) of FD & C Red No. 3 in Z-15-(T20)-3 were prepared. These colored zein fluids were added to the tablets in essentially the same manner as the uncolored zein fluids but failed to produce an evenly colored tablet. Initial addition of Z-15-(T20)-3 containing very small concentrations of FD & C Red No. 3, followed by subsequent coatings containing increasing dye concentrations failed to improve the uniformity of the coating color. The same negative results followed initial sealcoating of the tablets using shellac.

After varying dye concentrations and application techniques it was found that, per 10,000 commercial placebos (Pa), 50 ml. of Z-15-(T20)-3 containing 0.03% FD & C Red No. 3, added in a single continuous fine stream with twenty seconds of forced warm air applied immediately after all the tablets have been wetted by the coating fluid, produced the most desirable color. It is worth noting however, that the use of colored zein fluids, when applied in the same manner as the uncolored zein fluids, but with increasing concentrations of dye per application, can also result in a rather unique, strongly variegated coating, should this be desirable.

Visual Examination of Coated Tablets.—Random samples of the coated and uncoated tablets listed in Table II were examined for luster and homogeneity in the following manner. Evaluation of luster was performed in a darkened room using the reflectance of light from a 3-watt bulb on the 5X Aplanat Magnifier. The lens of the magnifier was removed for this inspection. The tablets were rated

TABLE I FORMULATIONS AND METHODS OF APPLICATION

Formula Number	Initial Tablets and Quantity Used	Blower Time ^a	Inter val ^b	1	2	3	4	5	6	7	8	9	10	11	12	Total
Z-15-(T20)-3 FD & C Red No. 3 0.03%	Pa-10M	20	50													59
Z-15-(T20)-3	AX-10M	20	2	15	20	15	15	10								80
Z-15-(A83)-3	Px-10M	20	2	15	20	15	15	15	10	10	15					135
Z-15-(A83)-3	AX-10M	20	3	15	20	15	15	15	15							100
Z-15	Pa-10M	20	2	15	15	15	10	10	10	10						85
Z-15-(S20)-3	Px-10M	30	1	20	10	10	10	10	10	10	10					90
Z-15-(S80)-3	Px-10M	30	1	20	10	10	10	10	10	10	10	10	10	10	10	150
Z-20-(T20)-1	AX-8M	20	2	15	15	15	10	15	10	10	10	10	10	10	10	110
Z-20-(T20)-5	Px-10M	20	2	15	15	15	10	15	15	15	10	10				100
Z-20-(T20)-10	Px-10M	20	2	15	15	15	10	15	15	15	10	10				80
Z-15-(T80)-3	Px-6M	30	1	20	10	10	10	10	10	10						200
Z-5-(T20)-3	Px-10M	20	2	20	20	20	20	30	20	20	20	30	10	10	10	180
Z-10-(T20)-3	Px-8M	20	2	20	20	20	20	20	20	10	10	20	10	10	10	150
Z-20-(T20)-3	Px-8M	20	2	20	20	20	20	10	10	10	10	10	10	10	10	115
Z-15-(T20)-1	Px-10M	20	2	15	15	15	10	15	10	10	10	10	10	10	10	105
Z-15-(T20)-5	Px-10M	20	2	15	15	15	15	15	10	10	10	15	15	15	15	145
Z-15-(T20)-10	Px-10M	20	2	15	15	15	15	10	10	15	15	15	15	15	15	145

^a Time in seconds during which forced warm air is directed on the tablets immediately after the coating fluid is evenly distributed^b Time interval in minutes between applications of coating fluid

TABLE II.—VISUAL EXAMINATION OF TABLETS

Formula Number	Initial Tablet	Luster ^a	Homogeneity ^b
Z-15-(T20)-3 FD & C Red No. 3-0.03%	Pa	2	4
Z-15-(T20)-3 waxed FD & C Red No. 3-0.03%	Pa	3	4
Z-15-(T20)-3	AX	1	2
Z-15-(T20)-3 waxed	AX	2	2
Z-15-(A83)-3	Px	3	2
Z-15-(A83)-3 waxed	Px	3	2
Z-15-(A83)-3 waxed	AX	3	1
Z-15	Pa	2	3
Z-15 waxed	Pa	4	3
Z-15-(S20)-3	Px	4	4
Z-15-(S80)-3	Px	4	4
Ferrous sulfate	.	4	4
Sodium salicylate	.	4	4
Ammonium chloride	.	4	4
Cascara sagrada	.	4	4
Ferrous gluconate	..	4	4
Uncoated Pa	.	4	4
Uncoated Px	.	3	3
Uncoated AX	.	0	0

^a Based on commercial placebo (4) as standard. Decrease in number represents decrease in luster^b Based on commercial placebo (4) as standard. Decrease in number represents decrease in homogeneity.

numerically for luster on a scale whereby the untreated commercial placebos (which possessed a very satisfactory luster without any coating) were rated as 4 and the untreated aspirin tablets having no luster rated as 0.

Homogeneity of the tablet surfaces was determined in a darkened room using the 5X Aplanat magnifying lens, the light source being the 3-watt bulb in the magnifier. Evaluation of homogeneity was numerical on a scale whereby untreated commercial placebos were rated as 4 (these tablets being very smooth and homogeneous in appearance). Values of 3 and 2 were assigned to tablets with decreasing homogeneity and a value of 1 for those tablets exhibiting a definite lack of homogeneity.

Infrared Heat Test.—Six each of the coated and uncoated tablets listed in Table II were selected at random and placed on the base plate of the infrared heat lamp, seven inches from the two 250-watt infrared lamps. The tablets were inspected at time intervals of 5, 10, 15, and 30 minutes, and 1, 2, 4, and 8 hours. Results of this test strongly indicate that zein coated tablets are more resistant to heat than the commercial sugar coated tablets and generally show little deleterious effects from the temperature employed. Zein coatings failed to protect the aspirin, which melted in every instance. All the zein coatings employed were free from the scorching effects of the heat. Waxed and unwaxed Pa colored with FD & C Red No. 3 began to fade after one-half hour exposure.

Durability (Shaking) Test.—Four-ounce glass French square bottles were filled to one-half capacity with coated and uncoated tablets as listed in Table II. These bottles were placed on their sides in the shaker and inspected at scheduled intervals for a total of two hours. This test is such that the bottles are shaken lengthwise through a total distance of approximately one inch three hundred times per minute.

During each examination of the tablets any powder which may have accumulated in the bottles during the test was removed to facilitate a more advantageous inspection. Results of these examinations indicate that zein coated tablets are more resistant to physical trauma than either the uncoated tablets or the commercially sugar coated tablets. Waxed zein coatings are more subject to chipping than the same unwaxed coatings.

Humidity Test.—Random samples of the tablets listed in Table III were put in uncovered Petri dishes which were then placed in a 250-ml. glass desiccator from which the desiccant had been removed and replaced with one inch of water. The tablets on each dish were in a single layer, thereby exerting only their own weight on the glass and not being compressed from above by other tablets. The dishes were filled to approximately three-fourths capacity to allow the tablets to slide freely when they were tilted at an angle. The Petri dishes used and the quantity of tablets employed were such that a maximum of visual inspection was possible with a minimum of epidermal contact. The conditions employed permitted a free movement of moist air equally over every tablet contained in the glass desiccator jar. The desiccator jar was securely closed but the Petri dishes contained therein were uncovered. The tablets were examined periodically for adherence to the glass dish which would indicate a softening of the coating due to the high humidity of the desiccator jar. Adherence values were assigned as described in Table III.

U. S. P. Tablet Disintegration Test.—Disintegration tests were performed on coated and uncoated tablets using the prescribed apparatus (10). Six each of the tablets listed in Table IV were tested in five replicates giving a total of five average disintegration values for each type tablet used. The average values are recorded in Table IV for disintegration in distilled water and artificial gastric juice as employed by Huyek (11).

TABLE IV.—DISINTEGRATION TEST

Coating Formula	Initial Tablet	Disintegration Time, Sec.	
		Distilled Water	Artificial Gastric Juice
Z-15-(T20)-3 FD & C			
Red No. 3-0.03%	Pa	46	39
Z-15-(T20)-3 waxed			
FDC & Red 3-0.03%	Pa	47	39
Z-15-(T20)-3	Ax	22	20
Z-15-(T20)-3 waxed	Ax	27	23
Z-15-(A83)-3	Px	98	93
Z-15-(A83)-3 waxed	Px	116	102
Z-15-(A83)-3 waxed	Ax	32	30
Z-15	Pa	59	45
Z-15 waxed	Pa	63	44
Z-15-(S20)-3	Px	105	93
Z-15-(S80)-3	Px	119	106
Uncoated Pa	..	49	39
Uncoated Px	..	108	106
Uncoated Ax	..	12	12

DISCUSSION

Varying concentrations of zein (5 to 20) in 91% isopropyl alcohol were employed as nonenteric tablet coatings. In most instances additives were combined with these fluids. The type and quantity of additives employed varied and ranged in concentration from 1 to 10%. Certain of these tablet coating formulas were chosen for testing based entirely upon the general appearance of the finished coated tablet. Fluids containing 5% and 10% zein were too thin, requiring larger quantities to cover the tablets adequately. Furthermore, the tablets are thus exposed to more zein solvent, thereby permitting possible tablet disintegration in the coating pan. Zein concentrations of 20% resulted in rough, uneven coatings. Additive concentrations of 10% resulted in a greatly prolonged drying time. Fluids containing 15% zein and 3% additive were sub-

TABLE III.—HUMIDITY TEST

Formula Number	Initial Tablet	Time, in Days*									
		1	2	3	4	7	8	9	11	14	
Z-15-(T20)-3 FD & C Red No. 3-0.03%	Pa	0	0	0	0	0	0	0	0	0	
Z-15-(T20)-3 waxed FD & C Red No. 3-0.03%	Pa	0	0	0	0	0	0	0	0	0	
Z-15-(T20)-3	Ax	4	6	6	8	6	6	6	6	6	
Z-15-(T20)-3 waxed	Ax	3	3	4	4	3	3	3	4	6	
Z-15-(A83)-3	Px	0	0	0	0	0	0	0	0	0	
Z-15-(A83)-3 waxed	Px	0	0	1	1	1	1	0	2	2	
Z-15-(A83)-3 waxed	Ax	0	0	2	3	3	4	4	5	6	
Z-15	Pa	0	0	0	0	0	0	0	0	0	
Z-15 waxed	Pa	0	0	2	2	1	0	0	2	0	
Z-15-(S20)-3	Px	0	0	0	0	0	0	0	0	1	
Z-15-(S80)-3	Px	0	0	0	0	0	0	0	0	0	
Ferrous sulfate	..	4	7	8	8	10	10	10	10	10	
Sodium salicylate	..	3	3	3	5	8	9	10	10	10	
Ammonium chloride	..	3	4	5	9	10	10	10	10	10	
Cascara sagrada	..	3	4	6	8	10	10	10	10	10	
Ferrous gluconate	..	2	3	3	3	8	10	10	10	10	
Uncoated Pa	..	0	0	0	0	0	0	0	0	0	
Uncoated Px	..	0	0	0	0	0	0	0	0	0	
Uncoated Ax	..	0	0	0	0	0	0	0	0	0	

* 0 adherence—all tablets slide readily when dish is tilted; 3 adherence—tablets slide only when dish is gently tapped with finger; 10 adherence—none of the tablets were released when dish is tapped as above; all required individual pressure to move them, leaving a portion of the tablet and coating remaining on the glass

mitted to testing because these concentrations appeared most efficacious with respect to uniformity and ease of coating. Total working time employing the prescribed concentrations of zein and additive was less than one-half hour for the actual coating procedure. At the end of this period the coating was dry and but for a characteristic odor of zein and isopropanol, the tablets were ready for packaging. This odor gradually dissipates when the tablets are exposed to air. The need for sealcoating, cooking kettles, and ovens is eliminated and the size and weight of the coated tablets is practically unchanged (1-5 mg weight gain). Zein coated tablets resisted effects of the durability, humidity and infrared heat tests to a greater degree than did the commercial sugar coated tablets. It would appear that aspirin presents an individual problem. Zein coatings completely mask the characteristic odor of acetic acid ordinarily emitted from aspirin tablets and remain on these tablets long enough to prevent the very familiar bitter taste of this drug from being experienced by the subject. Disintegration time is but slightly effected by the zein coatings and emblems, trademarks or grooves on a tablet surface are clearly visible through the coating.

From the design of this work it can be seen that no comparison between individual zein coating formulas was intended and that no such evaluations can be made from the accumulated data. It does appear, however, that zein can be well utilized as a noncenteric tablet coating material when applied in the form of a thin film.

REFERENCES

- (1) Clarkson, R., "Tablet Coating," Drug and Cosmetic Ind., New York, N. Y., 1951.
- (2) Cook, E. F., and Martin, E. W., "Remington's Practice of Pharmacy," 11th ed., Mack Publishing Co., Easton, Pa., 1956, p. 400.
- (3) Rowell, T. H., "The Art of Coating Tablets," T. H. Rowell, Baudette, Minn., 1949.
- (4) Gans, E. H., and Chavkin, L., THIS JOURNAL, 43, 483(1954).
- (5) Doerr, D. W., Serles, E. R., and Deardorff, D. L., THIS JOURNAL, 43, 433(1954).
- (6) Waksman, S. A., and Starkey, R. L., J. Bacteriol., 23, 405(1932).
- (7) Khigler, D., and Krehl, J., J. Nutrition, 41, 215(1950).
- (8) Koren, J. A., "Improved Methods of Tablet Coating," Thesis, University of Illinois, Chicago, 1948.
- (9) Villacorta, C. V., "Studies of Compressed Tablet Manufacture," Thesis, University of Illinois, Chicago, 1950.
- (10) "The United States Pharmacopeia," 15th rev., Mack Publishing Co., Easton, Pa., 1955, p. 937.
- (11) Huyck, C. L., THIS JOURNAL, Pract. Pharm. Ed., 7, 86(1946).

Book Notices

Methoden der organischen Chemie (Houben-Weyl), 4th ed. Vol. XI/1. Edited by EUGENE MULLER. George Thieme Verlag, Stuttgart, 1957. Price \$48.50 (subscription price \$44.55).

In Volume XI of this handbook, the methods of preparation and transformation of amines, lactams, quaternary ammonium compounds, and nitrogen compounds containing sulfur are discussed. Methods for the direct introduction of amino groups including methods involving addition reactions, reduction, condensation, the use of organometallic compounds, rearrangement, fission, and other special procedures are discussed quite in detail. Preparative methods for primary, secondary, and tertiary amines are described in a separate chapter and the properties and manipulation of ammonia are also discussed in another chapter dealing with this subject. Volume XI/1 is of the same high type of binding and format as the earlier volumes in this series.

Biochemistry of Some Peptide and Steroid Antibiotics. By E. P. Abraham. John Wiley & Sons, Inc., New York, 1957. ix + 96 pp. 12.5 x 18.5 cm. Price \$3.

This compilation of the 1957 Ciba Lectures in Microbial Biochemistry, at Rutgers University, by Dr. Abraham (Oxford), describes recent work on the bacitracins, the cephalosporins, and their structural and functional relationships to other antibiotics.

Unexpected Reactions to Modern Therapeutics Antibiotics. By Leo Schindler. Charles C. Thomas, Springfield, Ill., 1957. xii + 146 pp. 12.5 x 18.5 cm. Price \$3.

This survey deals first with unexpected reactions to antibiotics, describing signs and symptoms due to these reactions. The antibiotics discussed are: penicillin, streptomycin-dihydrostreptomycin, chloramphenicol, tetracyclines, antibiotic-resistant bacteria, neomycin, erythromycin, bacitracin, fumagillin, novobiocin, cycloserine, and polymyxin. The management of unexpected reactions is considered. References are given at the end of each chapter, and a general subject index is appended.

Atomic Energy in Agriculture. By William E. Dick. Philosophical Library, New York, 1957. 13.5 x 22 cm. Price \$6.

This book describes how atomic energy may speed up plant breeding and the production of new varieties of plants, and how atomic energy can be used in other ways in agriculture.

Dental Practitioners' Formulary 1957. By the Joint Formulary Committee. The British Medical Association, London, 1957. 49 pp. 10 x 16 cm. Price 3s.

This third edition of the Dental Practitioners' Formulary is intended for use by dentists in the British National Health Service.

Scientific Edition
**JOURNAL OF THE
AMERICAN PHARMACEUTICAL
ASSOCIATION**

VOLUME XLVII

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**Kinetics of the Degradation of Isoamyl Nitrite
in Ampuls I.***

**Nature of Reaction Products and Influence of Temperature, Oxygen,
Alcohol, Water, and Acid on the Overall Rate**

By MARTIN H. YUNKER, DALE SZULCZEWSKI, and TAKERU HIGUCHI

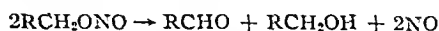
Results of a kinetics study on the rate of degradation of both laboratory and commercial lots of isoamyl nitrite in sealed ampuls are presented. The reaction appears to be autocatalytic with an effective heat of activation of 20 kcal./mole. Commercial ampuls containing roughly 0.33 ml. of the ester explode on developing from 7-12 ml. of by-product gases. Mass spectrometric and gas chromatographic analyses show that these consist largely of nitrogen, nitrous oxide, carbon dioxide, and traces of nitric oxide and carbon monoxide. Results obtained suggest that the first three gases are produced by a secondary reaction between nitric oxide and aldehyde. The liquid products appear to be a mixture consisting of at least twelve components including carboxylic ester, carboxylic acid, isoamyl alcohol, aldehyde, and isoamyl nitrate. The influences of water, acid, alcohol, oxygen, and certain additives on the overall degradative rate are also given.

THE PRESENT STUDY is concerned with the results of an investigation on the problem of deterioration of isoamyl nitrite (amyl nitrite, U. S. P.) on storage. The nitrite ester on standing undergoes spontaneous decomposition, yielding both gaseous and liquid degradation products. This instability is manifested in commercial amyl nitrite ampuls by a steady increase in internal pressure leading eventually to explosive destruction of the glass container. In this work, experimental data were obtained showing the chemical nature of the end products, the rate of pressure development, the influence of temperature, water, and oxygen on the rate, and finally the catalytic effect of acid for the liquid phase, thermal breakdown reaction of the ester especially in sealed ampuls.

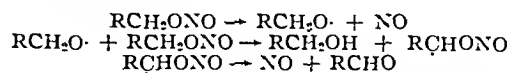
Much of the past studies on the decomposition

reactions of the nitrite esters have dealt with vapor phase, high temperature systems.

The thermal decomposition of methyl, ethyl, *n*-propyl, isopropyl, and *n*-butyl nitrites in the vapor phase has been studied by Steacie and co-workers (1) at 170-200°. Under these conditions the major overall reaction in each case appeared to be:

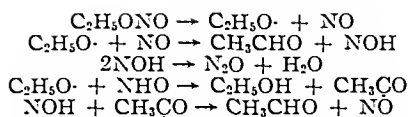


Rice and Rodowskas (2) have proposed the following mechanism for the vapor phase reaction of alkyl nitrites:



In a more recent study of the vapor phase reaction of ethyl nitrite, however, Levy (3) disagrees with Rice's mechanism, and finds that the following scheme agrees better with his kinetic data.

* Received February 13, 1958, from the School of Pharmacy, University of Wisconsin, Madison



In each of the above studies, nitric oxide was found to be the principal gaseous product while alcohol and aldehyde were the predominant liquid decomposition products. In addition, however, Levy found appreciable nitrous oxide, and had reason to believe water is formed.

The only study of the liquid phase decomposition of a nitrite ester was made by Kornblum and Oliveto (4) on 2-octyl nitrite at 100°. In this study, the authors continually flushed the reacting system with carbon dioxide and collected the gaseous products above 50 per cent potassium hydroxide. The principal products of the reaction were found to be nitric oxide, 2-octanone, and 2-octanol as would be expected by the previously postulated mechanisms for the vapor phase reaction. In addition, they found significant amounts of nitrogen, nitrous oxide, and carbon dioxide as gaseous products, and several acids and esters of various chain lengths as liquid products. The formation of these additional compounds was thought to arise from the cleavage of 2-octanone by nitric oxide followed by esterification of 2-octanol by the acids formed.

CHEMICAL NATURE OF PRODUCTS

There seems to have been no earlier attempts made to establish the chemical nature of the products resulting from decomposition of isoamyl nitrite in ampuls. As a phase of the present investigation, both the liquid and the gaseous products formed during the breakdown of the ester in sealed ampuls were analyzed.

Gaseous Products.—Gases isolated from commercial ampuls of the ester ranging in age from two to four years were analyzed qualitatively and quantitatively by both mass spectrometry and by gas chromatography. A typical analysis using gas chromatography (5) of the gaseous phase found in old amyl nitrite ampuls (Burroughs Wellcome & Co., Lot 143, date of manufacture November 26, 1951) is shown in Table I. The accuracy of the method is indicated by the data in Table II which gives the

TABLE I.—PERCENTAGE COMPOSITION OF GAS FOUND IN OLD AMYL NITRITE AMPULS^a

Sample	N ₂	N ₂ O	CO ₂	NO	CO
1	50	33	15	Trace	Trace
2	48	35	12	Trace	Trace
3	48	33	19	Trace	Trace
4	48	34	16	Trace	Trace
5	49	37	14	Trace	Trace
Av.	49 ^b	34 ^b	15 ^b		

^a Burroughs Wellcome & Co., Lot 143, date of manufacture November 26, 1951.

^b Refers to per cent by volume.

results of analysis of a mixture of similar gases of known composition. From this it is seen that the gas phase consisted of a mixture of nitrogen, nitrous oxide, carbon dioxide, and traces of nitric oxide and carbon monoxide. The presence of the large amount of nitrogen and the near absence of nitric oxide were rather surprising in view of the results obtained by earlier workers. No indication of any oxygen was found despite the fact that the ampuls were sealed under air. Because of this apparent ambiguity several experiments were conducted to illustrate possible routes by which nitric oxide could disappear from the system with the production of nitrogen and the other significant nitrogen containing gas, nitrous oxide.

The observation of Kornblum and Oliveto (4) that nitric oxide is capable of reacting with 2-octanone at 100° to yield liquid oxidation products and nitrogen, nitrous oxide, and carbon dioxide led the authors to investigate the possibility of a similar reaction with an aldehyde at a lower temperature. The results of analyzing the gas phase obtained from the reaction of *n*-butyraldehyde and nitric oxide at 50° are seen in Table III. From these data it is seen that in only one case was there evidence of a reaction. In this case the resulting gaseous products were similar to those observed to be present in old amyl nitrite ampuls.

TABLE II.—ANALYSIS OF A GASEOUS MIXTURE OF KNOWN COMPOSITION BY GAS CHROMATOGRAPHY (5)

Gas	O ₂	N ₂	%	N ₂ O	CO ₂
Known	19	38		23	19
Found ^a	17	37		24	21

^a Average of five determinations.

TABLE III.—ANALYSIS OF THE GAS PHASE RESULTING FROM REACTION OF NITRIC OXIDE AND *n*-BUTYRALDEHYDE AT 50°

Time, Hr.	N ₂	NO	%	N ₂ O	CO ₂
0	2	98		0	0
24.5	2	98		0	0
47.5	67	0		22	11
96.0	2	98		0	0
211.0	8	92		0	0
288.0	6	94		0	0

Wt. *n*-butyraldehyde, 22 mg.; volume ampul, 2.5 ml.; pressure filled under, 74 cm. Hg; temperature filled under, 25°.

Since the behavior of this reaction with time is ambiguous, it was thought possible that some catalytic species was inadvertently introduced into the particular ampul which showed complete destruction of nitric oxide. Since contamination by a small quantity of air would not be unlikely, another experiment was performed in identical manner as before with the exception that the nitric oxide was deliberately contaminated with oxygen. As seen from Table IV in the presence of even the smallest concentration of oxygen, the nitric oxide reacts with the aldehyde completely in the relatively short incubation time of twelve hours. In all probability the catalytic species is either nitrogen dioxide or its dimer. This observation is substantiated by the

TABLE IV.—ANALYSIS OF THE GAS PHASE RESULTING FROM THE REACTION OF NITRIC OXIDE WITH A TRACE OF NITROGEN DIOXIDE AND *n*-BUTYRALDEHYDE AT 50°

Time, Hr	O ₂ Added	N ₂	% NO	N ₂ O	CO ₂
0	0	2	98	0	0
12	2	68	0	20	12
12	4	64	0	25	11

Wt *n*-butyraldehyde, 22 mg., volume ampul, 2.5 ml., pressure filled under, 74 cm Hg., and temperature filled under, 25°

work of Kuhn (7) who observed a similar phenomenon in the case of nitric oxide oxidation of benzaldehyde.

The ability of nitric oxide to react with aldehyde, a reducing agent, prompted investigation into another possible nitrogen producing reaction, namely that of nitric oxide and the ester itself. By referring to Table V one sees that it appears as if nitric oxide will react with isoamyl nitrite itself under these conditions with the production of nitrogen, nitrous oxide, and carbon dioxide. These results are not entirely clear due to the likely presence of an aldehyde. However, it is felt that the production of nitrogen is too rapid to be explained entirely on the basis of a possible aldehyde-nitric oxide reaction.

In order to ascertain whether nitric oxide would be found in a significant amount if the gaseous products were continually removed from the reacting system, an experiment similar to that of Kornblum and Oliveto was performed. The results are summarized in Table VI. The presence of potassium hydroxide precluded any analysis for carbon dioxide. The increase of nitric oxide from the trace found in the case of the closed system to 40%, as indicated, is significant.

The possibility of nitrous oxide, by virtue of its relatively low decomposition temperature and the exothermic nature of its decomposition, acting as a nitrogen producing intermediate was investigated

TABLE V.—ANALYSIS OF THE GAS PHASE RESULTING FROM THE REACTION OF ISOAMYL NITRITE AND NITRIC OXIDE AT 50°

Time, Hr	N ₂	% NO	N ₂ O	CO ₂
0	2	98	0	0
23	12	86	0	2
51	22	72	1	5
72 5	37	59	< 1%	3
120 0	65	< 1%	12	18
190 0	70	Trace	14	16

Ampul volume, 2.5 ml., wt isoamyl nitrite, 35 mg., temperature at filling, 24°, and pressure filled at, 74 cm Hg.

TABLE VI.—ANALYSIS OF THE GAS PHASE RESULTING FROM DEGRADATION OF 100 ML. OF ISOAMYL NITRITE AT 70° IN REACTING SYSTEM CONTINUALLY FLUSHED WITH CARBON DIOXIDE GASEOUS PRODUCTS COLLECTED ABOVE 50 PER CENT POTASSIUM HYDROXIDE

Time, hr.	48
Nitrogen, %	58
Nitric Oxide, %	40
Nitrous Oxide, %	1

Ampuls containing 35 mg. of isoamyl nitrite and filled under 76 cm. Hg pressure of nitrous oxide were incubated for 240 hours at 50°. Two additional experiments were conducted in which the isoamyl nitrite was replaced with isoamyl alcohol and *n*-butyraldehyde. In each case the results indicated that no reaction occurred.

Liquid Products.—Infrared absorption spectra of the liquid phase isolated from ampuls which were kept at 70° over a two week period showed a constant increase in carbonyl and organic nitrate absorption. During the first seven or eight days of storage, two peaks were present in the carbonyl region at 5.8 μ and 5.86 μ , but only a single peak at 5.82 μ was present after two weeks. This would indicate that a transformation from acid to ester was occurring during the decomposition. The addition of oxygen was found to considerably increase the amount of nitrate and acid formed during the first several days of storage.

Infrared absorption spectra of the fraction obtained from a fractional distillation of a sample kept for two weeks at 80° showed ester to be the predominant product, with acid, aldehyde, alcohol, and nitrate also present in lesser amounts. Characterization of the low and high boiling fractions by chemical means identified isoamyl isovalerate, isovaleryl aldehyde, and isovaleric acid as being present in the liquid mixture. Water, which was present as an insoluble layer in the original decomposition mixture, accounted for approximately 8–10% of the total products.

As previously mentioned, an experiment in which the ester was allowed to decompose in an open, flushed system was conducted. Infrared absorption spectra of samples from this experiment collected over a twelve day period of storage at 70° showed that no significant degradation had occurred. This would indicate that the mechanism proposed by Steacie is not primarily responsible for the products of the degradation of isoamyl nitrite in the liquid phase in sealed ampuls.

A more thorough study based on vapor phase chromatographic separation of the reaction products has shown that at least 12 products are formed in the liquid phase. The results of this investigation will be published shortly.

INFLUENCE OF TEMPERATURE

The temperature dependency of the reaction was studied over the range of 50–80°. Figure 1 shows the increase in gas volume per ampul to be linear with time over the range studied. The measurements were carried out on commercially prepared ampuls which were approximately one year old. The amount of gas present in the ampuls at the beginning of the experiment varied from 4 to 6 cc., consisting largely of gases produced during storage. This volume of gas, representing from 15 to 20% degradation, probably corresponded to the rapid phase of the reaction. This part of the reaction is responsible for the final, linear portion of the curve shown in Fig. 4 for ampuls filled under air. The average 10° temperature coefficient corresponds to approximately 2.4. The average heat of activation as determined from the Arrhenius plot (Fig. 2) for this portion of the degradative reaction was 20 kcal./mole.

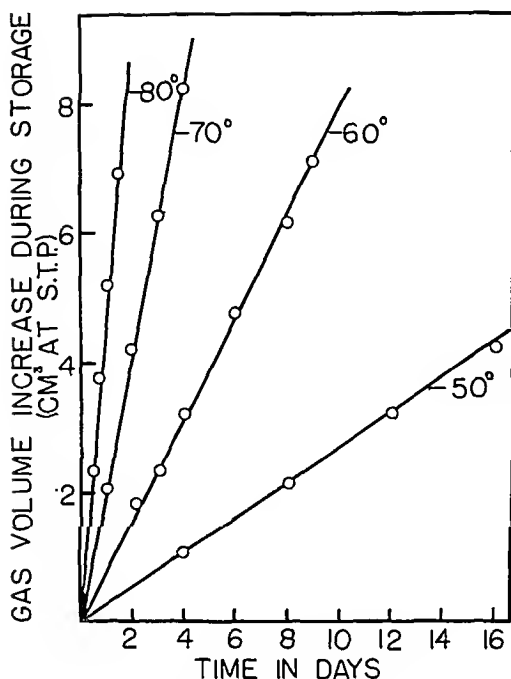


Fig. 1.—A plot showing the effect of temperature on the rate of gas production per ampul. The commercial ampuls, containing 0.33 ml. of isoamyl nitrite and filled under air, were approximately one year old.

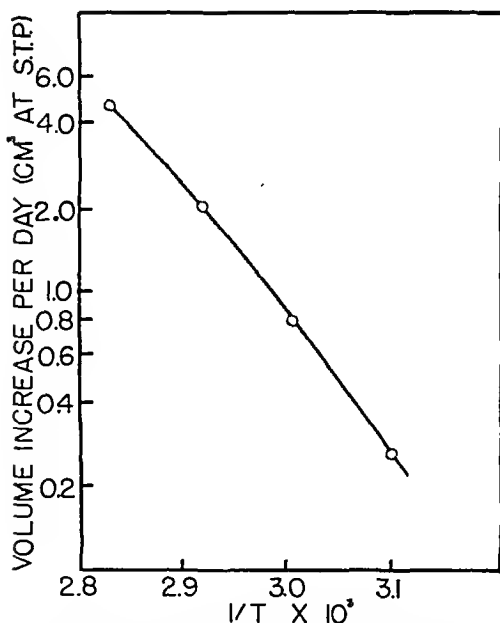


Fig. 2.—An Arrhenius type plot for the decomposition of isoamyl nitrite in commercial ampuls. The ampuls were approximately one year old and contained 0.33 ml. of isoamyl nitrite filled under air.

In considering the rate of decomposition which was found for isoamyl nitrite in sealed ampuls, it becomes apparent that the reaction involved is

different from that proposed by Steacie for the thermal decomposition of simple nitrite esters in the vapor phase. Steacie found that the rate of decomposition could be expressed in the form of the following rate equation

$$k = 1.39 \times 10^{14} e^{-37,700/RT}$$

for the overall reaction:



It can be calculated from this rate equation that a period of twenty-four years would be required to give 10% decomposition at 70°. In the present study, it can be shown that 4.17 ml. of gas represents a minimum of 10% decomposition in ampuls containing 0.5 ml. of isoamyl nitrite, since one mole of the ester will decompose to form $\frac{1}{2}$ mole of nitrogen or nitrous oxide. If the initial rate is considered in the plot of gas volume against time for isoamyl nitrite alone (Fig. 3, under air and vacuum), it can be seen that sixteen days is required to produce 4.2 ml. of gas. Considering the final rate, only 2.6 days is necessary to give this amount of gas. This very great difference in rates of decomposition found in the two studies can only be accounted for by a difference in reactions. This is also evident from the considerably lower heat of activation which was found for the degradation of isoamyl nitrite in ampuls.

INFLUENCE OF OXYGEN, WATER, ALCOHOL, AND VOLUME OF ISOAMYL NITRITE PER AMPUL

A second phase of this study included an investigation of the influence of oxygen, water, alcohol, and

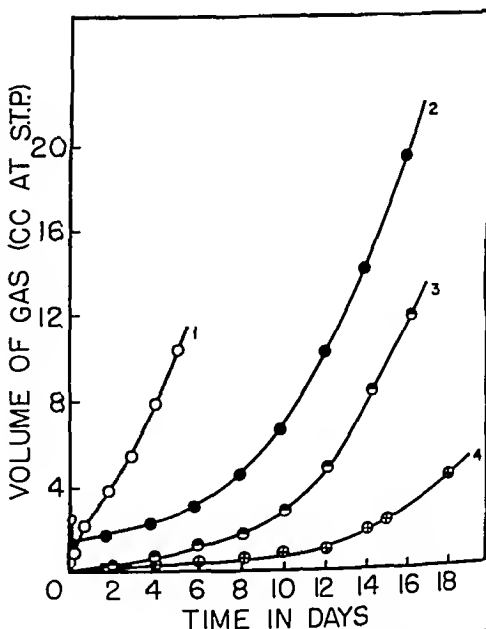


Fig. 3.—A plot showing the effect of oxygen and sodium phosphate (solid) on the rate of gas formation at 70° in ampuls containing 0.5 ml. of isoamyl nitrite. (1) Ampuls filled under 1.7 atmospheres of oxygen; (2) ampuls filled under air; (3) ampuls filled under vacuum; (4) ampuls filled under vacuum with 1% w/v trisodium phosphate added.

volume of nitrite ester per ampul on the rate of gas formation. It was felt that this type of information would provide valuable clues concerning the causes of instability of the nitrite ester in ampuls.

Effect of Oxygen.—It was noted previously that analysis of the gases present in ampuls which had been filled under air showed no oxygen present, indicating that oxygen was probably reacting during the breakdown. For this reason, it was felt desirable to investigate the effect of oxygen on the rate of gas formation.

The data from this study are summarized in Fig. 3. When ampuls are filled under vacuum, there is a considerable period during which the formation of gas is relatively slow. After the eighth day at 70°, there is a very rapid increase in the rate, and it finally becomes linear with time. The shape of the curve suggests that autocatalysis is taking place during the reaction. It is highly probable that the first portion of the curve represents the period during which the catalytic species is slowly formed. After the concentration of the species reaches a sufficient level, the catalytic effect becomes very pronounced, accounting for the final rapid rate of gas formation. The linearity of the limiting rate with time might represent a balancing effect between the formation and disappearance of the catalytic species.

The same type of curve was obtained when the ampuls were filled under air. However, the time required to reach a rapid rate of gas formation was considerably less, which suggests that oxygen is capable of forming the catalytic species.

When the ampuls were filled under oxygen, nearly all of the oxygen had disappeared at the end of one hour. Then the rate of gas formation became rapid without showing any induction period. The oxygen evidently caused a sufficient concentration of the catalytic species to be formed immediately, resulting in an initial rapid rate. It is interesting to note that the final slope of the curves for the three cases are quite similar.

Since nitric oxide appears to be an intermediate product of the reaction, oxygen apparently converts this gas to nitrogen dioxide. The nitrogen dioxide may be a catalytic species, or may react further to form another catalytic species. The fact that a catalytic effect was noted even when the ampuls were filled under vacuum indicates that oxygen is not necessary to form the catalytic species.

Effect of Water.—The fact that nitrite esters would be expected to undergo hydrolysis quite readily in the presence of water, especially if acid is present, prompted a study of the effect of water on the reaction. As can be seen from Fig. 4, 0.8% water approximately doubles the rate of gas formation in the early stages of degradation. This does not seem like a very large effect if hydrolysis of the ester were primarily responsible for the breakdown. The fact that the effect is not as large as might be expected if hydrolysis were a factor could possibly be explained by the slight solubility of water in isoamyl nitrite. Also, acid may be present only in very small quantity during the first part of the degradation, and thus, may not appreciably catalyze the hydrolysis.

Effect of Dehydrating Agents.—In view of the effect of water, it was expected that dehydrating agents might decrease the degradation. Figure 4

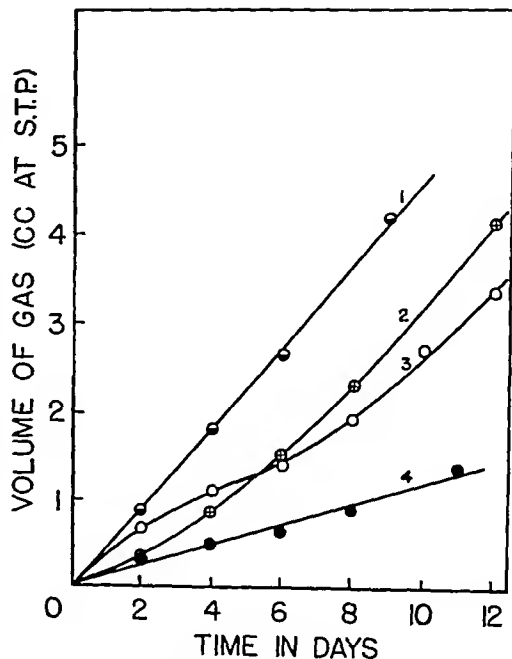


Fig. 4.—A plot showing the effect of water, calcium sulfate, and magnesium sulfate on the rate of gas formation in ampuls containing 0.5 ml. of isoamyl nitrite filled under vacuum and kept at 60°. (1) 5% w/v anhydrous magnesium sulfate; (2) 0.8% v/v water; (3) 10% w/v anhydrous calcium sulfate; (4) isoamyl nitrite alone.

shows surprisingly that both anhydrous calcium sulfate and magnesium sulfate increased the rate of gas formation about to the same extent as water. This apparent ambiguity is in all probability caused by some unknown catalytic effect of the dehydrating agents upon the reaction. Possibly, the agents possess sufficient acidity to catalyze the breakdown of the ester.

Effect of Ethanol and Nitrobenzene.—It can be seen from Fig. 5 that alcohol does not have any significant effect on the rate. This experiment was conducted at 51°, and therefore the rate of degradation was relatively slow with no autocatalytic effect noted. Isoamyl nitrite is known to undergo a transesterification reaction with ethyl alcohol to form ethyl nitrite. This reaction could increase the formation of gas only if ethyl nitrite decomposes at a much faster rate than isoamyl nitrite. Since only 1% ethyl alcohol was added, the amount of ethyl nitrite produced would necessarily be small.

Nitrobenzene did not show any significant effect on the rate of gas formation as shown in Fig. 5.

Effect of Volume of Isoamyl Nitrite per Ampul.—When the rates of gas formation at different volumes of isoamyl nitrite from Fig. 6 are plotted against volumes of isoamyl nitrite per ampul, a straight line is obtained (Fig. 7). This shows that the rate of gas production per ampul is directly proportional to the volume of isoamyl nitrite per ampul. Since the total volume of the ampuls was always 2.10 ml., the void space above the liquid decreased as the volume of the liquid was increased. The results indicated that the gas produced by degradation is

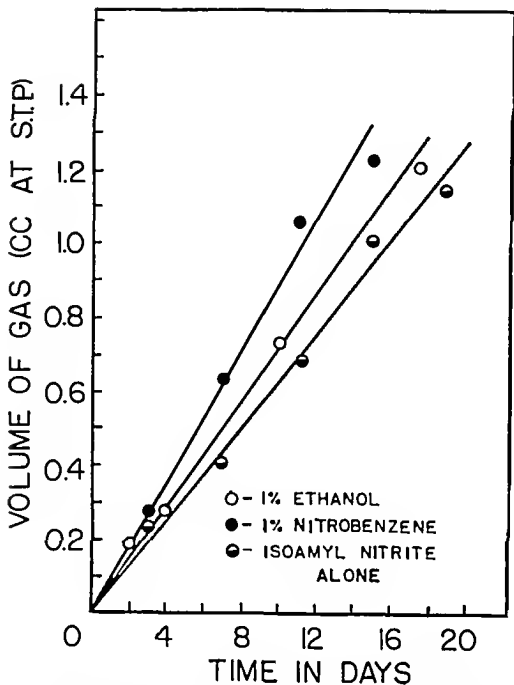


Fig. 5.—A plot showing the effect of nitrobenzene and ethyl alcohol on the rate of gas formation per ampul at 51°. The ampuls were filled under vacuum and contained 0.5 ml. of isoamyl nitrite.

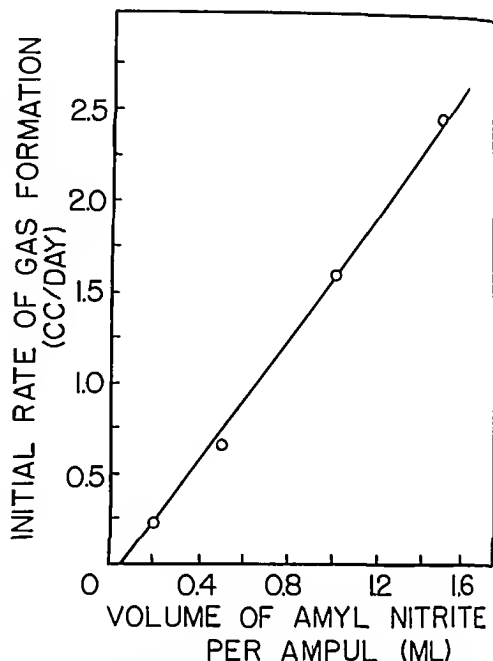


Fig. 7.—A plot showing the rate of gas production per ampul to be directly proportional to the volume of isoamyl nitrite per ampul.

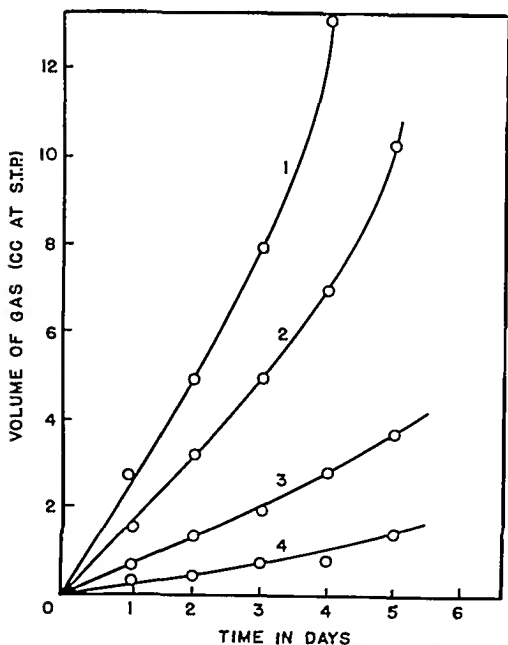


Fig. 6.—A plot showing the effect of varying the volume of isoamyl nitrite per ampul on the rate of gas production at 70°. The ampuls were filled under vacuum. (1) 1.5 ml. per ampul; (2) 1.0 ml. per ampul; (3) 0.5 ml. per ampul; (4) 0.2 ml. per ampul.

largely the result of reactions occurring in the liquid phase. This does not necessarily mean that gas phase reactions are absent since the amount of isoamyl nitrite in the gas phase as compared to that in the liquid phase is so small as to make any gas phase reaction appear negligible as far as contribution to the gaseous products is concerned. This study indicates that the practical shelf life of amyl nitrite ampuls is dependent upon the volume of isoamyl nitrite added to the ampuls.

INFLUENCE OF ACID

Since it has been shown by other workers that acid may be a product of the reaction (6), the effect of addition of strong acid to isoamyl nitrite was investigated. Trichloroacetic acid in concentrations varying from 0.16 to 2% was added to ampuls containing 0.5 ml. of the ester.

It can be seen from Fig. 8 that the reaction is very sensitive to acid. When acid is added in concentrations of 0.5 to 2%, the rate of initial gas formation is very rapid, but gradually decreases to a constant value. The nature of the curves suggests that different reactions are responsible for the initial and final stages of degradation. It can be seen from Fig. 9 that the initial rate is proportional to the square of the acid concentration. This suggests that two molecules of acid are involved in a reaction with the nitrite ester. It can be seen from Fig. 10 that the terminal rate is directly proportional to the acid concentration which would indicate that only one molecule of acid is involved in that reaction.

The behavior in the presence of strong acid during the initial phase might be accounted for by the acid catalyzing a transesterification reaction between

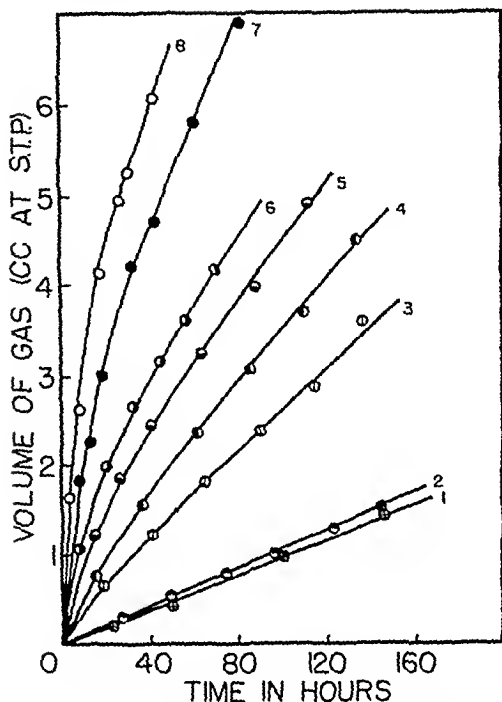


Fig. 8.—A plot showing the effect of varying the concentration of trichloroacetic acid on the rate of gas production in ampuls containing 0.5 ml. of isoamyl nitrite filled under vacuum and kept at 60°. (1) Isoamyl nitrite alone; (2) 0.17% acid; (3) 0.46% acid; (4) 0.65% acid; (5) 0.87% acid; (6) 1.09% acid; (7) 1.51% acid; (8) 2.03% acid.

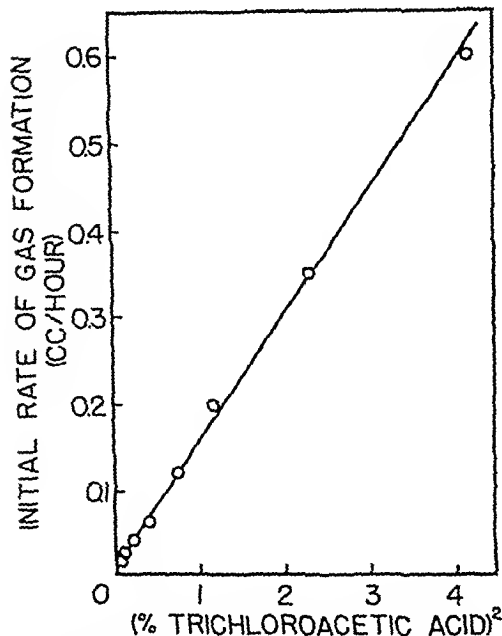


Fig. 9.—A plot showing the initial rate of gas production per ampul to be proportional to the square of the concentration of acid added to isoamyl nitrite.

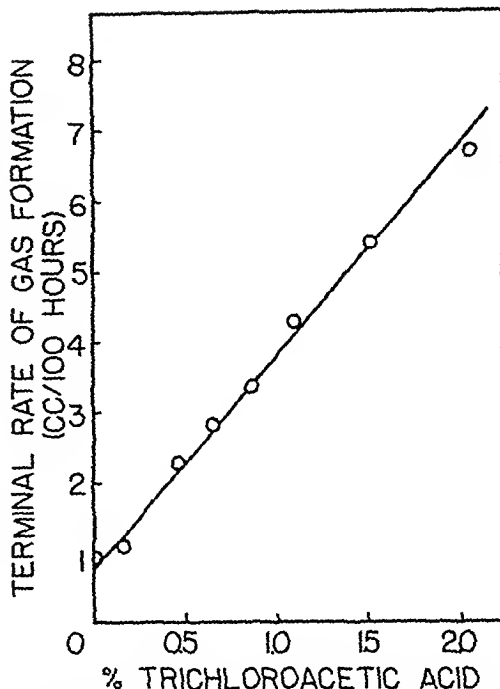
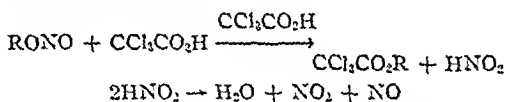


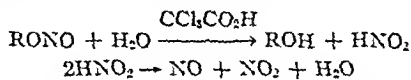
Fig. 10.—A plot showing the terminal rate of gas production per ampul to be directly proportional to the concentration of acid added to isoamyl nitrite.

itself and the nitrite ester. This could be represented as follows:



In a previous section, it was shown that the nitric oxide or nitrogen dioxide probably reacts further with aldehyde to produce a mixture of gases containing nitrogen as the principal component.

In view of the fact that water has been shown to be a product, it is evident that trichloroacetic acid can catalyze the hydrolysis of the ester, as shown below;



The above results show that if a strong acid were formed during the degradation, it would in all probability show a catalytic effect on the breakdown reaction. The importance of eliminating the presence of acid in commercial amyl nitrite is also pointed to by the results of this study.

EXPERIMENTAL

Reagents.—The nitric oxide was prepared by the nitrometer reaction since commercial nitric oxide is often contaminated with small amounts of nitrogen dioxide. The isoamyl nitrite was Fisher reagent grade distilled under vacuum prior to being used. A sample of *n*-butyraldehyde was obtained from a commercial lot by fractional distillation on

plate Oldershaw fractionating column, analysis of this sample revealed that it was better than 98% pure. The remainder of the chemicals were of the analytical reagent grade.

Experimental Procedure for Analysis of Gaseous Products.—Liquid samples were placed in calibrated ampuls by means of a standardized dropper. The ampul was then placed in a bath of dry ice-acetone and evacuated. The various gases in question were allowed to enter the evacuated ampul through a system of stopcocks and the freezing bath withdrawn in order to allow the ampul to warm up to room temperature. When temperature equilibration was reached the pressure was adjusted to atmospheric. The stopcock connecting the gas source to the ampul was then turned off and the freezing bath replaced. By using a very fine flame from a blowpipe the ampul was sealed off from the remainder of the system.

The gas analysis was performed by gas chromatography using a silica column as previously described.

Gasometric Analytical Procedure.—An analytical method which permitted the volumes of gas produced in ampuls by degradation to be measured proved the most suitable for this study. A schematic drawing of the apparatus is shown in Fig 11. The method consists essentially of breaking the ampuls in an evacuated chamber and then sweeping the decomposition gases into a semimicro nitrometer (Pregl type) with carbon dioxide. The nitrogen containing decomposition gases displace the 50% potassium hydroxide in the nitrometer whereas the carbon dioxide is completely absorbed by the alkali. The volumes of gas are read in the graduated part of the nitrometer at existing barometric pressure and temperature, and are subsequently converted to volumes at standard conditions. Before breaking each ampul, a small amount of carbon dioxide was passed into the nitrometer to see if the air had been completely swept out of the system. The breaking chamber was then evacuated and the ampul broken by lowering a polyethylene coated iron bar onto it with an electromagnet. The gas was next

swept into the nitrometer and the final volume read after the bubbles of gas completely dissolved again in the potassium hydroxide.

The solubility of nitrous oxide in 50% potassium hydroxide was determined since this gas is appreciably soluble in water. The gas was found to be, for practical purposes, insoluble under conditions of the analytical procedure.

Ampuls for Gasometric Study.—Commercially available ampuls made of resistant glass, and having a total volume of approximately 2 ml were used. In all of the experiments, except for when the effect of changing the volume of isoamyl nitrite was studied, 0.5 ml of isoamyl nitrite was introduced into each ampul with a 1.0 ml volumetric pipet. The ampuls were sealed at a calibration mark to have a total volume of 2.10 ml.

The ampuls were filled under vacuum by connecting the ampul directly to a vacuum system. The ampuls containing isoamyl nitrite were immersed in a dry ice-acetone mixture during evacuation to prevent loss of the ester and then sealed while remaining in the mixture. In filling ampuls under oxygen, the gas was allowed to displace the air in the ampuls while they were immersed in a dry ice-acetone mixture. They were subsequently sealed.

Commercially made ampuls containing 0.33 ml. of amyl nitrite were used for the temperature dependency study. These ampuls were approximately one year old. Ampuls were taken from a single commercial lot for each temperature studied. The volumes of gas from at least four ampuls were averaged for the final reading at each time interval. In all of the other experiments, the volumes from two ampuls were averaged for each reading.

Decomposition of Samples in Gasometric Study.—Each batch of ampuls was placed in a light resistant, wide mouth bottle, and the bottle was placed in a thermostatically controlled bath set to the desired temperature. Samples were withdrawn at suitable intervals and were stored in the refrigerator until used.

A blank consisting of ampuls filled with isoamyl nitrite alone was run for each experiment since the rate of gas formation was found to differ between blanks.

REFERENCES

- (1) Steacie, E. W. R., "Atomic and Free Radical Reactions," Reinhold Publishing Corp., New York, N. Y. 1956, p. 141.
- (2) Rice, F. O., and Rodowskas, L. L., *J. Am. Chem. Soc.* 57, 350 (1935).
- (3) Levy, J. B., Paper presented at the 126th meeting of A. C. S., New York, N. Y., Sept. 12-17, 1954.
- (4) Kornblum, N., and Oliveto, E. P., *J. Am. Chem. Soc.* 71, 226 (1949).
- (5) Szulcowski, D., and Higuchi, T., *Anal. Chem.* 29, 1541 (1957).
- (6) Buchi, J., and Kuer, V., *Pharm. Acta Helv.* 15, 59 (1940).
- (7) Kuhn, P., Paper presented at the 126th meeting of A. C. S., New York, N. Y., Sept. 12-17, 1954.

SCHEMATIC DRAWING OF VOLUME MEASURING APPARATUS

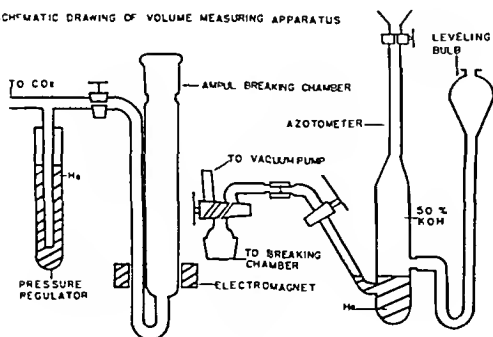


Figure 11.

Kinetics of Degradation of Amyl Nitrite Ampuls II.*

Stabilization

By MARTIN H. YUNKER† and TAKERU HIGUCHI

It is shown that the stability of amyl nitrite ampuls can be greatly increased by the addition of various substances—including potassium carbonate (solid), trisodium phosphate (solid), magnesium oxide (solid), pyridine (dissolved), and diphenylamine (dissolved). The mechanism of stabilization appears to involve behavior of these agents as sinks for nitrogen dioxide and/or nitric acid produced during the breakdown. The degree of stabilization achieved suggests that the shelf life of the nitrite ester can be readily prolonged as much as tenfold as compared to present commercial products.

SINCE isoamyl nitrite (amyl nitrite U. S. P.) undergoes serious degradation in ampuls, with formation of considerable gas, the present study was undertaken to investigate possible methods of stabilization. No previous attempt appears to have been made to stabilize alkyl nitrites.

In a previous publication (1), it was suggested that the relatively rapid formation of gas in amyl nitrite ampuls was due to the autocatalytic nature of the reaction, a catalytic species being progressively produced during the degradation process. Since it was shown that the rate was substantially increased by addition of a strong acid, the possibility arose that an acid might be accelerating the decomposition. Investigations were, therefore, conducted to determine the effect of alkaline substances on the rate of gas formation in amyl nitrite ampuls.

EXPERIMENTAL

Reagents.—The isoamyl nitrite used for this study was reagent grade material which was vacuum distilled immediately before use, the middle fraction from the distillate being employed.

Procedure.—The experimental procedure and the method of analysis were essentially the same as those used for Part I of this series.

The agents used in this study fell into four categories: (A) a strong base (triethylamine); (B) solid acid absorbents (potassium carbonate, sodium phosphate, magnesium oxide, and sodium bicarbonate); (C) a weak base (pyridine); and (D) those amines which are neutral, but might be expected to react with nitric acid (diphenylamine, *para*-nitromethylaniline). The effects of the agents were studied in 1 to 2% w/v concentration added to ampuls containing 0.5 ml. of isoamyl nitrite. Generally the ampuls were filled under vacuum unless otherwise indicated. The water-soluble salts were added to isoamyl nitrite by dissolving them in a measured volume of distilled water, and then introducing a suitable aliquot

of this solution into the ampul. The water was subsequently evaporated off, and the isoamyl nitrite then added. A blank consisting of ampuls filled with isoamyl nitrite alone was run for each experiment.

Analysis of the Carbonate.—The residue from several ampuls of isoamyl nitrite to which 5% carbonate had been added was separated by filtration, and washed thoroughly with ether to extract all of the isoamyl nitrite. Qualitative tests were used to determine if nitrite and nitrate were present. Total nitrite and nitrate were determined quantitatively by the nitrometer method (2), and nitrite alone by the permanganate method (3).

RESULTS

Strong Base.—The effect of the addition of 1% v/v triethylamine to isoamyl nitrite on the rate of gas formation per ampul is shown in Fig. 1. It is seen that the rate is very rapid at first, and then decreases as the reaction proceeds. Since the rate is considerably greater than with isoamyl nitrite alone, it appears that strong bases are not suitable for stabilization of isoamyl nitrite.

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If a 10 degree temperature coefficient of 2.4 is used for the reaction (1), the 19 day period of stabilization with 2% carbonate shown in Fig. 3 is equivalent

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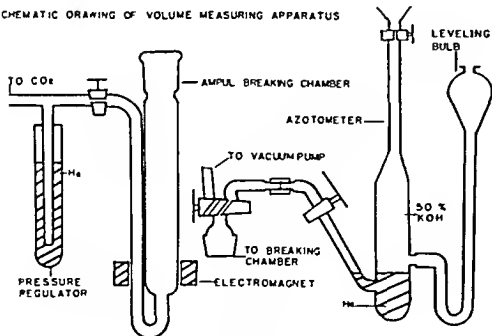


Figure 11.

REFERENCES

- (1) Steacie, E. W. R., "Atomic and Free Radical Reactions," Rhenhold Publishing Corp., New York, N. Y., 1946, p. 141.
- (2) Rice, F. O., and Rodowskas, E. L., *J. Am. Chem. Soc.*, **57**, 350 (1935).
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Stabilization

By MARTIN H. YUNKER† and TAKERU HIGUCHI

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Procedure.—The experimental procedure and the method of analysis were essentially the same as those used for Part I of this series.

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Solid Acid Absorbents.—The data in Fig. 2 show that when 2% w/v potassium carbonate is added to the isoamyl nitrite ampuls, the increase in volume of gas per ampul is much less than that for isoamyl nitrite alone. These results indicate that isoamyl nitrite can be stabilized in ampuls by the addition of alkaline salts which are capable of reacting with an acidic, catalytic species formed during the degradation. It might be expected that the ability to neutralize the acid, and thus stabilize the ester, would depend upon the amount of the alkaline salt added. This is shown to be true by the data in Fig. 3, from which it can be seen that 0.1% carbonate was very slightly effective whereas 2% carbonate was very effective in stabilizing the isoamyl nitrite. As the concentration of the carbonate was increased, the induction period became longer, and finally with 2% carbonate, no catalytic effect appeared in the curve.

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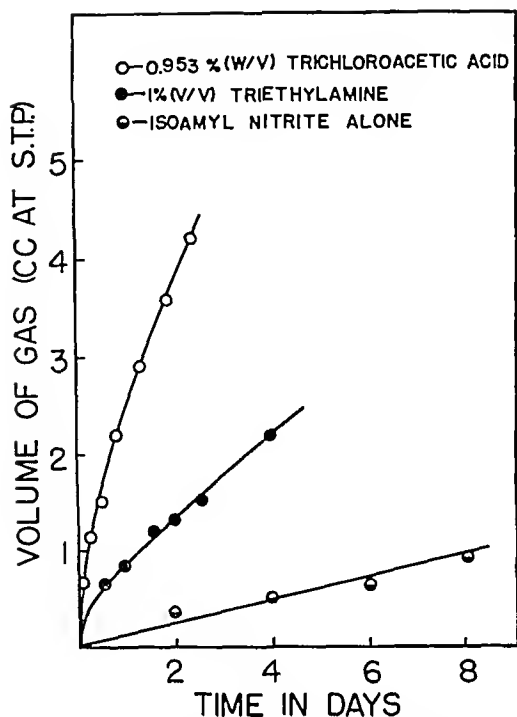


Fig. 1.—A plot showing the effect of triethylamine and trichloroacetic acid on the rate of gas formation per ampul at 60°. The ampuls contained 0.5 ml. of amyl nitrite filled under vacuum.

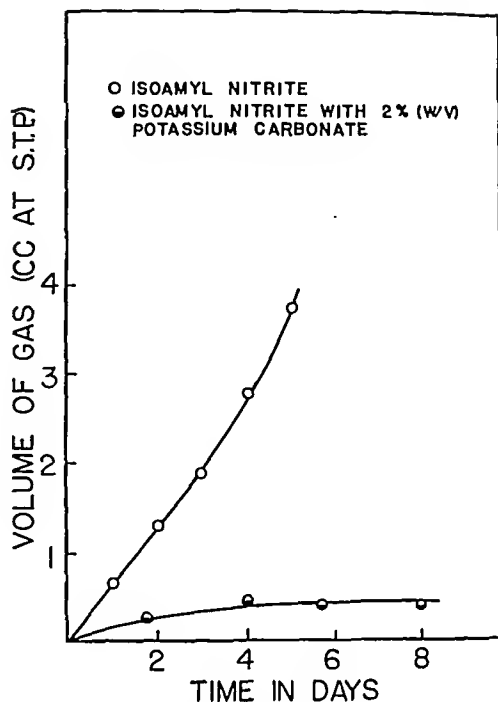


Fig. 2.—A plot showing how the addition of potassium carbonate (solid) to amyl nitrite affects the rate of gas production per ampul at 70°. The ampuls contained 0.5 ml. of amyl nitrite filled under vacuum.

to 2 years at room temperature. The volume of gas (1.46 cc.) produced during this period is much less than that amount (7 to 8 cc.) found necessary to cause commercial ampuls to explode. It should be noted also that commercial ampuls contain only 0.33 ml. of isoamyl nitrite, thus the volume of gas produced in them would be less than in this experiment where the ampuls contained 0.5 ml. It was shown in the previous article that the rate of gas production was directly proportional to the volume of isoamyl nitrite in the ampuls.

Sodium phosphate was also found to stabilize isoamyl nitrite in ampuls against gas formation, but to a slightly lesser degree than carbonate (Fig. 4). Magnesium oxide was more effective than sodium phosphate, perhaps because it is more alkaline. It can be seen in Fig. 5 that 2% w/v sodium bicarbonate substantially reduces the volume of gas per ampul. However, it is the least effective of the above acid absorbents, probably because it is also the least alkaline.

Since the above studies were conducted at 70°, it was decided to test the effectiveness of alkaline salts at a lower temperature. Four batches of ampuls containing: isoamyl nitrite filled under vacuum, isoamyl nitrite with 2% Na_3PO_4 filled under vacuum, isoamyl nitrite with 2% Na_3PO_4 filled under air, and isoamyl nitrite with 2% K_2CO_3 filled under vacuum were heated at 50° for 12 weeks. The results are summarized in Table I. They show that potassium carbonate is more effective at a lower temperature, having completely stopped the production of gas during a 12-week period. Sodium phosphate is seen to have substantially reduced the

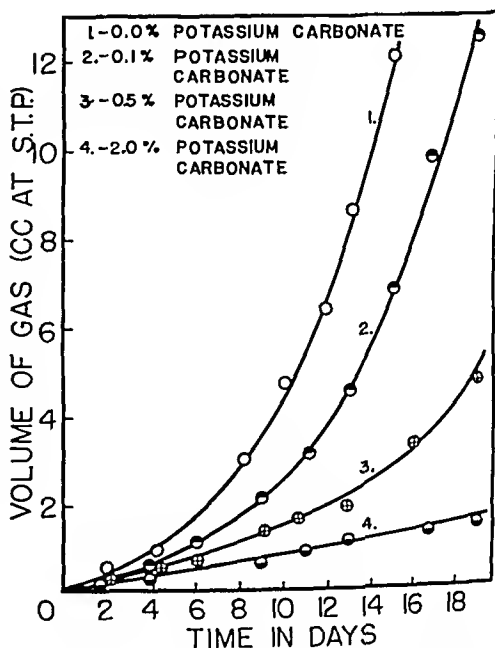


Fig. 3.—A plot showing the effect of varying the concentration of potassium carbonate on the rate of gas production per ampul of amyl nitrite at 70°. The ampuls contained 0.5 ml. of amyl nitrite filled under vacuum.

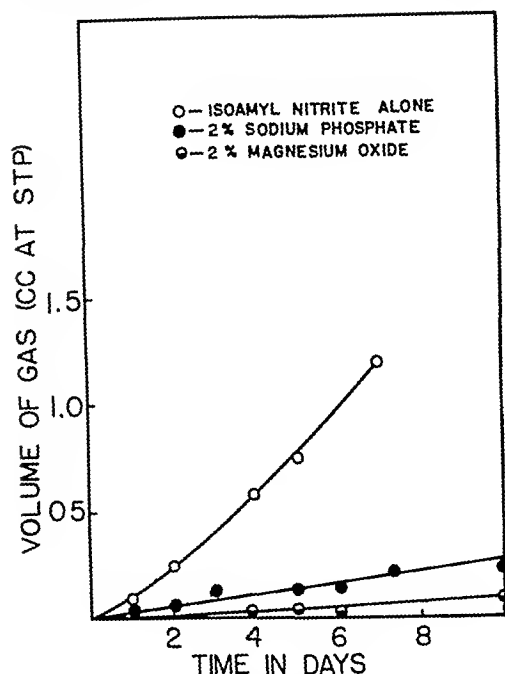


Fig 4—A plot showing the effect of trisodium phosphate (solid) and magnesium oxide (solid) on the rate of gas formation in ampuls containing 0.5 ml of amyl nitrite filled under vacuum

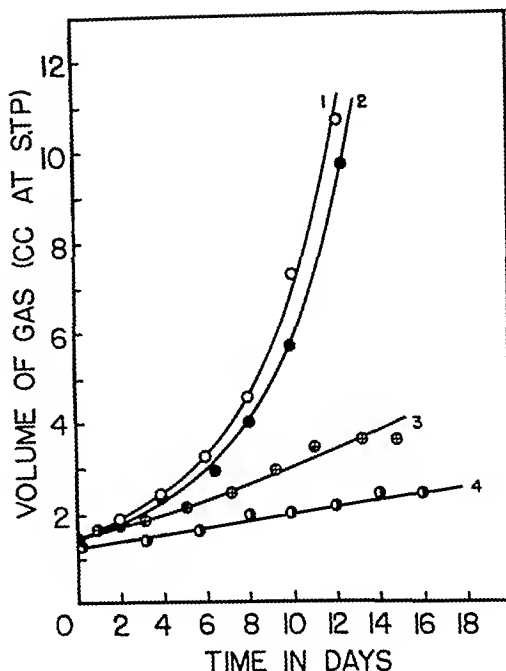
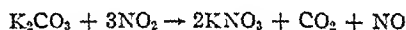


Fig 5—A plot showing how the addition of *p*-nitromethyl aniline (dissolved), pyridine (dissolved), and sodium bicarbonate (solid) affects the rate of gas production per ampul at 70°. All ampuls contained 0.5 ml of amyl nitrite filled under air. 1, 1% *p*-nitromethyl aniline, 2, isoamyl nitrite alone, 3, 2% sodium bicarbonate, 4, 2% pyridine

amount of gas, both in ampuls filled under vacuum and under air

The residue from ampuls stabilized with potassium carbonate responded positive to qualitative tests for nitrite and nitrate. Results of a quantitative analysis of the residue for nitrite and nitrate showed that 23 moles of nitrate were formed for each mole of nitrite, and that 40% of the 25 mg of carbonate in each ampul had been neutralized. Since one mole of isoamyl nitrite will react to form one mole of nitrite or of nitrate, it can be calculated that 3.8% of the ester had been decomposed by the above route. In considering that the experiment was run at 80° for 14 days, this is a relatively small amount of degradation. The nitrate found by the analysis could possibly be accounted for by a reaction between nitrogen dioxide and carbonate as reported by Addison and Lewis (4). This would be represented by the following equation



However, nitrate might also be formed by neutralization of nitric acid. The nitrite is most probably formed by neutralization of nitrous acid by the carbonate. These results definitely suggest that nitric acid or nitrogen dioxide is the catalytic species formed during the degradation.

Pyridine.—It was desirable to determine what effect a weak organic base might have on the reaction since it was previously shown that a strong organic base increased the degradation. It can be seen from Fig 5 that the addition of 2% v/v pyridine to amyl nitrite ampuls greatly decreases the volume of gas formed after a considerable period of storage at 70°. As with potassium carbonate, the pyridine has completely eliminated the autocatalysis

TABLE 1—THE VOLUMES* OF GAS FORMED AT 50° IN AMPULS CONTAINING 0.5 ML OF AMYL NITRITE AFTER ADDITION OF 2% (W/V) OF K_2CO_3 AND Na_3PO_4

Storage Time	0 wk	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk
Amyl Nitrite—under vacuum	0	1.18	2.98	4.35	11.6	All ampuls had broken	
2% Na_3PO_4 added—under vacuum	0	0.57	0.98	1.10	1.57	1.82	2.78
2% Na_3PO_4 added—under air	1.24	1.39	1.62	1.78	2.14	2.42	2.75
2% K_2CO_3 added—under vacuum	0	0.09	0.09	0.15	0.19	0.16	0.04

* Volumes of gas in ml corresponding to 0° at 760 mm of mercury pressure

noted for isoamyl nitrite alone. Since pyridine would not be expected to be nitrated by nitrogen dioxide, it appears that it may stabilize the ester by neutralizing nitric acid. In support of this theory, Addy and Mebeth (5), attempting nitrosation of pyridine with isoamyl nitrite, isolated pyridine nitrate from a mixture of the two which had been allowed to stand for a few days. The fact that pyridine exhibits a favorable influence whereas triethylamine did not is probably due to pyridine being a much weaker base, and thus, not being able to cause cleavage of the ester.

Diphenylamine.—Since diphenylamine has been used for the stabilization of nitrocellulose, it seemed advisable to investigate its effect on the reaction. From Fig. 6 it can be seen that 1% w/v diphenylamine essentially stops the formation of gas during 14 days of storage at 70°. Upon addition of the amine, the color of isoamyl nitrite was changed to a deeper yellow, and a precipitate formed after several days of heating. The yellow precipitate melted over a considerable range of temperature, and did not show any improvement after recrystallization. It gave a red color with sodium hydroxide solution characteristic of nitro compounds. The solid was chromatographed according to the procedure of Schroeder (6). After repeated rechromatography, two colored compounds were separated on the column which were identified as 2,4'-dinitrodiphenylamine and 4,4'-dinitrodiphenylamine by comparing their ultraviolet spectra (Fig. 7) with those listed for dinitro derivatives of diphenylamine by Schroeder (7). The location of the maxima and minima agree very well; however, the ratios of D max./D

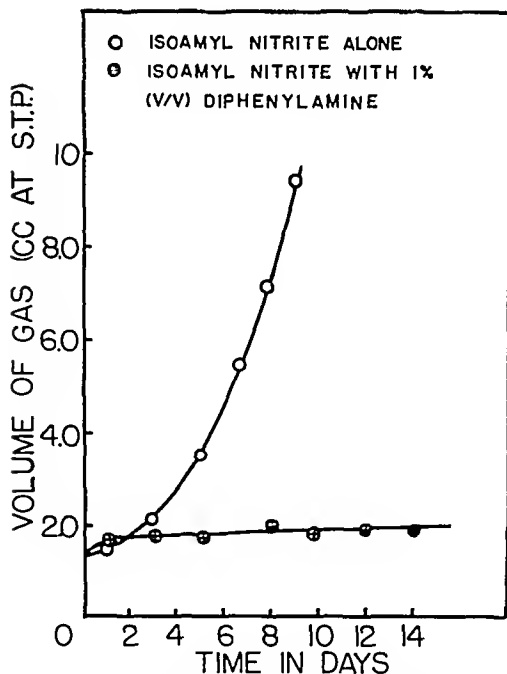


Fig. 6.—A plot showing how the addition of diphenylamine (dissolved) to amyl nitrite affects the rate of gas production per ampul at 70°. The ampuls contained 0.5 ml. of amyl nitrite filled under air.

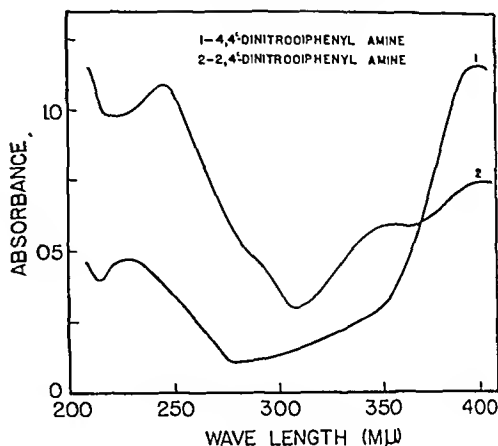


Fig. 7.—Ultraviolet spectra of the nitro derivatives of diphenylamine formed when diphenylamine is added to amyl nitrite.

min. of our compounds were lower, indicating that they were less pure. The melting points of our compounds were also slightly lower:

	Listed	Found
2,4'-dinitro D. P. A.	220°	217°
4,4'-dinitro D. P. A.	216°	210°

The color reactions were identical to those reported by Ashdown and Davis (8).

These results indicate that diphenylamine is probably nitrated by nitric acid formed during the reaction. It is interesting to note that Ashdown and Davis were able to isolate the same two dinitro derivatives from nitrocellulose which had been stabilized with diphenylamine. Schroeder reports that diphenylamine stabilizes nitrocellulose by continuously combining with nitrogen acids and oxides which arise during the decomposition, and thus, decreases the autocatalytic effect of these products on the further decomposition of the nitrocellulose. There are two possible mechanisms by which the dinitro derivatives might be formed: (A) the classical method which consists of a series of nitrosations, rearrangements, and oxidations, or (B) a series of nitrosations, denitrosations, and nitrations as proposed by Schroeder (6). The second scheme does not involve any rearrangements or formation of C-nitroso compounds, but only nitrosation of the diphenylamine itself. Although we did not isolate the N-nitroso compound, it is probably formed during the reaction since alkyl nitrites are known to be nitrosating agents.

Para-Nitromethylaniline.—Gallagher and Pincus (9) reported that N-alkyl nitro anilines which contain an alkyl group such as Me, Et, iso-Pr, and also contain a nitro in either the 3 or 4 position on the ring were superior to diphenylamine for stabilizing nitrocellulose. They stated that the compounds were sufficiently neutral and absorb nitric acid and highly oxidized nitrogen atoms. Therefore, it was thought that these compounds might also prove useful for stabilizing isoamyl nitrite.

The results in Fig. 5 surprisingly show that 1% w/v of *p*-nitromethylaniline increased the volume of gas produced per ampul. Why this compound should not stabilize isoamyl nitrite while diphenyla-

mine does is not easily explained. Possibly the two compounds react with nitric acid by different mechanisms and this in some way accounts for their different behavior toward isoamyl nitrite. It is noteworthy that Gallagher and Pincus stated that diphenylamine reacts with nitric acid and nitrogen dioxide to produce nitric oxide whereas the N-alkyl nitro anilines rapidly absorb nitrogen dioxide and nitric acid without reducing them.

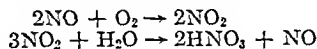
Confirmation of Stabilization by Infrared Spectral Analysis.—Although it has been shown that carbonate will stop the gas formation in amyl nitrite ampuls, the question existed whether any changes were occurring in the liquid phase. For this reason, the infrared absorption spectrum of isoamyl nitrite which had been heated for 12 weeks at 50° in the presence of carbonate, and had shown no gas formation, and the spectrum of isoamyl nitrite alone that had been heated for 8 weeks at 50° were obtained. In comparing these with the spectrum of pure isoamyl nitrite, it was found that no significant change had occurred in the spectrum of the stabilized isoamyl nitrite. The spectrum of the unstabilized control sample, as expected, indicated that it had undergone extensive degradation, with considerable ester and nitrate formation. These results show that when isoamyl nitrite is stabilized against gas formation in ampuls, it does not undergo any significant decomposition.

DISCUSSION

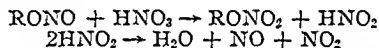
The results of these studies indicate that the addition of solid acid absorbents such as potassium carbonate, or of diphenylamine, to isoamyl nitrite may prove useful in preventing loss of amyl nitrite ampuls due to gas formation. This would be of particular advantage when the ampuls are stored for a prolonged period.

It has been shown that nitric acid which is formed during the decomposition probably catalyzes the degradation of isoamyl nitrite in ampuls. In the previous article, it was noted that nitric oxide was formed during the reaction, and also that oxygen had a catalytic effect on the degradation. Water was

also reported to be a product of the reaction. In considering these facts, the formation of nitric acid might be explained by the following scheme:



This could account for the catalytic effect of oxygen previously reported. However, since catalysis was also noted when the ampuls were filled under vacuum, it appears that another reaction is involved by which nitric acid can be formed. It was shown previously that all of the oxygen disappeared in a short time from the ampuls, so the above scheme could account for only an initial formation of nitric acid. Further work on the decomposition needs to be done to clarify this phase. The effect of nitric acid on the reaction is probably similar to that of trichloroacetic acid previously noted. It can catalyze the hydrolysis of isoamyl nitrite to the alcohol and nitrous acid with the latter breaking down further to nitric oxide and nitrogen dioxide. There is also some evidence that the acid might be catalyzing a transesterification reaction between itself and isoamyl nitrite,



since it has been shown in the previous article that an organic nitrate is continuously formed during the reaction.

REFERENCES

- (1) Higuchi, T., Yunker, M. H., and Szulcowski, D. H., *This Journal*, **47**, 613 (1958).
- (2) Vogel, A. I., "Quantitative Inorganic Analysis," 2nd ed., Longmans, New York, N. Y., 1951, p. 285.
- (3) *Ibid.*, p. 808.
- (4) Addison, C. C., and Lewis, J., *J. Chem. Soc.*, 1319 (1953).
- (5) Addy, C. W., and Mcbeth, A. K., *ibid.*, 109, 754 (1916).
- (6) Schroeder, W. A., Malmberg, E. W., Fong, L. C., Trueblood, K. N., Landerl, J., and Hoerger, E., *Ind. Eng. Chem.*, **41**, 2818 (1949).
- (7) Schroeder, W. A., Wilcox, P. E., Trueblood, K. N., and Dekker, A. O., *Anal. Chem.*, **23**, 1740 (1951).
- (8) Ashdown, A. A., and Davis, T. L., *Ind. Eng. Chem.*, **17**, 674 (1925).
- (9) Gallagher, J. A., and Pincus, I., U. S. pat. 2,696,430, Dec. 7, 1954.

The Reserpine, Rescinamine, and Deserpidine Content of Rauwolfia Roots*

By DANIEL BANES, ALBERT E. H. HOUK, and JACOB WOLFF

The alkaloids reserpine, rescinnamine, and deserpidine occurring in the roots of various *Rauwolfia* species are separated by column chromatography, and determined by ultraviolet spectrophotometry.

SEVERAL METHODS have been published on the determination of reserpine in rauwolfia root. Kidd and Scott (1) isolated the alkaloid by countercurrent distribution, and analyzed the

fractions by ultraviolet spectrophotometry. Their assay values on small representative samples from 5-Kg. batches of powdered root were in excellent agreement with the actual yield of crystalline reserpine from the entire batch. Sakai and Merrill (2) employed paper ionophoresis for the separation of rauwolfia alkaloids, and corrected the observed ultraviolet absorption of the reserpine fraction for absorbing contaminants. Dechene (3) extracted the feebly basic alkaloids of *Rauwolfia serpentina* from dilute sulfuric acid

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solution with chloroform, treated the extracted alkaloids with hydrogen peroxide in acid solution and calculated the fluorescing substances indiscriminately as reserpine.

Carol and his collaborators (4) have analyzed *Rauwolfia serpentina* for both reserpine and rescinnamine. They separated a feebly basic alkaloidal fraction by liquid liquid partition chromatography on columns hydrolyzed the alkaline esters extracted the acids obtained, and determined trimethoxybenzoic and trimethoxycinnamic acids simultaneously by ultraviolet spectrophotometry. The same group (5) later published a colorimetric procedure for the "reserpine rescinnamine group alkaloids" in *Rauwolfia serpentina* based on the nitrite test of Szalkowski and Mader (6), and showed that the two methods yielded concordant results. The nitrite procedure was studied collaboratively in several laboratories and is being considered for adoption as the official assay procedure for the National Formulary (7).

Deserpidine, an alkaloid first found in *Rauwolfia canescens* (8) has been stated (9) to occur as a minor constituent in several *Rauwolfia* species. However, no quantitative data have been published on the concentration of this potent alkaloid in the roots of any species. Hayden Ford and Houk (10) have employed column partition chromatography for the complete separation of deserpidine, reserpine, and rescinnamine in eluates suitable for spectrophotometric measurements. We have adapted their procedure to extracts of *Rauwolfia serpentina*, *Rauwolfia canescens*, *Rauwolfia vomitoria*, and *Rauwolfia heterophylla*.

EXPERIMENTAL

Finely powdered rauwolfia root (5-10 Gm) was extracted in a Soxhlet extractor with alcohol for four hours, and the extract was diluted to 100 ml. The reserpine rescinnamine group alkaloids in a 20 ml aliquot were separated by the procedure of Banes, *et al* (5). The combined chloroform extracts were mixed with 30 ml of alcohol, and evaporated to dryness under vacuum at a temperature below 70°. The residue was dissolved in about 1 ml of chloroform and the solution was transferred with the aid of several 0.5 ml portions of chloroform to a beaker containing 2 Gm of siliceous earth. After thorough mixing, the solvent was evaporated under vacuum at room temperature, and the residue was incorporated in a chromatographic column, as described by Hayden, *et al* (10). Consecutive 10-ml cuts were examined to locate the fractions desired. Those fractions containing high concentrations of deserpidine, reserpine, or rescinnamine were combined and diluted, if necessary, to facilitate spectrophotometric measurements.

Powdered extracts of roots were dissolved in

chloroform, and suitable aliquots were mixed with siliceous earth, vacuum dried and analyzed chromatographically. Tablet samples were powdered, and a quantity equivalent to 5 Gm of root was analyzed according to the procedure for powdered whole root.

DISCUSSION

The elution pattern of deserpidine, reserpine, and rescinnamine from rauwolfia root extracts was consistent with the observations of Hayden and her collaborators (10). The forerun, containing small quantities of substances with the ultraviolet absorption spectra of 3 dehydroreserpine, aporphine, and reserpine, was usually 100-120 ml. Deserpidine appeared within the next 90 ml of eluate, reserpine in the next 150-200 ml cut, and rescinnamine in the following 300 ml. These volumes varied with the concentrations of the individual alkaloids. The ultraviolet absorption of the fractions intervening between the chief alkaloids showed a slight background absorption, equivalent to less than 2% of the reserpine or rescinnamine content in *Rauwolfia serpentina*. Deserpidine occurs in this species in much smaller proportions than the other alkaloids and the background absorption is accordingly of greater significance in its determination.

The data in Table I on the chromatographic analysis of various rauwolfia preparations show that deserpidine constitutes less than 3% of the total of reserpine, rescinnamine, and deserpidine in the ten authentic samples of *Rauwolfia serpentina* root analyzed. Reserpine concentrations in the whole root varied from 0.10 to 0.16%, with the rescinnamine content about 0.4 those values. In *Rauwolfia canescens* root the average concentration of both deserpidine and reserpine was approximately 0.07%, and rescinnamine was absent. No rescinnamine, and only small proportions of deserpidine were found in *Rauwolfia heterophylla*, while *Rauwolfia vomitoria* contained relatively large quantities of both rescinnamine and reserpine. These findings are in accord with the observations of earlier investigators.

Table II compares the total reserpine plus rescinnamine values obtained by the present method for *Rauwolfia serpentina* roots with reserpine rescinnamine group alkaloids assays by the nitrite colorimetric procedure. The two sets of values for the powdered roots show an excellent correlation, with the nitrite values greater by a factor of 1.11-1.18. These figures demonstrate that the nitrite assays for reserpine rescinnamine content of *Rauwolfia serpentina* roots, include a small quantity of other chromogenic alkaloids. The tablet analyses showed greater variation between the two methods. However, both methods indicated correctly the composition of Sample 11, an uncoated tablet fabricated to contain 88 mg of Sample 4 (*Rauwolfia serpentina* powdered root) and 14 mg of excipients per tablet. Moreover, both methods agreed that Sample 13 contained slightly more of the active alkaloids than the known Sample 11, that Samples 12 and 13 were weakest and equivalent to each other, and that Sample 14 was intermediate in strength.

Although the present method yielded analytical data for reserpine and rescinnamine in several root samples almost identical with the results obtained by the earlier indirect hydrolytic procedure (1)

TABLE I—CHROMATOGRAPHIC ANALYSES OF RAUWOLFIA PREPARATIONS

Sample	Description	Mg per 100 Gm or per 1,000 Tablets		
		Deserpidine	Reserpine	Rescinnamine
1	<i>R. Serpentina</i> powdered root	5	158	57
2	<i>R. Serpentina</i> powdered root	6	143	62
3	<i>R. Serpentina</i> powdered root	3	141	66
4	<i>R. Serpentina</i> powdered root	4	131	67
5	<i>R. Serpentina</i> powdered root	5	128	56
6	<i>R. Serpentina</i> powdered root	5	107	48
7	<i>R. Serpentina</i> powdered root	5	115	44
8	<i>R. Serpentina</i> powdered root	4	103	41
9	<i>R. Serpentina</i> powdered root	4	98	45
10	<i>R. Serpentina</i> powdered root	4	97	35
11	<i>R. Serpentina</i> Tablets 88 mg ^a	3	119	60
12	<i>R. Serpentina</i> Tablets 100 mg	6	80	23
13	<i>R. Serpentina</i> Tablets 100 mg	5	76	28
14	<i>R. Serpentina</i> Tablets 100 mg	4	90	27
15	<i>R. Serpentina</i> Tablets 100 mg	4	124	64
16	<i>R. Serpentina</i> powdered extract ^b	910	33,500	20,000
17	<i>R. Serpentina</i> powdered extract ^b	1,100	33,400	17,900
18	<i>R. Serpentina</i> powdered extract	32	823	364
19	<i>R. Serpentina</i> powdered extract	24	268	168
20	<i>R. Canescens</i> powdered root	40	65	0
21	<i>R. Canescens</i> powdered root	82	70	0
22	<i>R. Canescens</i> powdered root	66	86	0
23	<i>R. Heterophylla</i> powdered root	5	87	0
24	<i>R. Heterophylla</i> powdered root	2	27	0
25	<i>R. Vomitoria</i> powdered root	5	201	41
26	<i>R. Vomitoria</i> powdered root	4	127	79

^a Fabricated to contain 88 mg of Sample 4 (*R. serpentina* powdered root) and 14 mg excipients^b Alleged to contain 30–35% reserpineTABLE II—ANALYSES OF *R. serpentina* PREPARATIONS

Sample ^a	Chromatographic Reserpine and Rescinnamine, mg/100 Gm	Reserpine Rescinnamine Group Alkaloids (5), mg/100 Gm
1	215	243
2	205	233
3	207	232
4	198	227
5	184	204
6	155	179
7	159	177
8	144	166
9	143	162
10	132	156
11	179	201
12	103	138
13	104	137
14	117	158
15	188	219

^a Sample numbers correspond to those in Table I

some discrepancies were encountered. In the case of one root, reserpine and reserpine were 19 and 12% higher, respectively, by the indirect method than by the chromatographic method. The present procedure is probably more reliable, since it isolates the alkaloids sought, and determines them individually by direct measurements.

SUMMARY

A procedure for the chromatographic separation of reserpine, rescinnamine, and deserpidine has been utilized for the determination of these alkaloids in various *Rauwolfia* species. Analytical data for *Rauwolfia serpentina* powdered root show excellent correlation with assay values for reserpine-rescinnamine group alkaloids obtained by the nitrite colorimetric procedure.

REFERENCES

- (1) Kidd, D. A. A. and Scott, P. G. W. *J. Pharm. and Pharmacol.*, **9**, 176 (1957).
- (2) Sakai, E. H., and Merrill, F. J., *THIS JOURNAL*, **43**, 709 (1954).
- (3) Dechene, F. *ibid.* **44**, 657 (1955).
- (4) Carol, J., Banes, D., Wolff, J., and Fallscheer, H. O., *ibid.* **45**, 200 (1956).
- (5) Banes, D., Wolff, J., Fallscheer, H. O., and Carol, J., *ibid.* **45**, 708 (1956).
- (6) Szalkowski, C. R. and Mader, W. J., *ibid.* **45**, 613 (1956).
- (7) *Drug Standards* **25**, 63 (1957).
- (8) Stoll, A., and Hofmann, A., *J. Am. Chem. Soc.*, **77**, 1071 (1955); Schlittler, E., Ulshofer, P. R., Pandon, M. L., Huat, R., and Dorfman, L., *Experientia*, **11**, 64 (1955); Slater, I. H., Rathbun, R. C., Henderson, F. G., and Neuss, N., *Proc. Soc. Exptl. Biol. Med.*, **88**, 293 (1955).
- (9) MacPhailamy, H. B., Dorfman, L., Huebner, C. F., Schlittler, E., and St. Andre, A. F., *J. Am. Chem. Soc.*, **77**, 1071 (1955).
- (10) Hayden, A. L., Ford, I., and Houk, A. F. H., *THIS JOURNAL*, **47**, 157 (1958).

The Uniformity of Distribution of Phosphorus Compounds in Tablet Matrices Using Radioactive Tracer Techniques*

By GILBERT S. BANKER, JOHN E. CHRISTIAN, and H. GEORGE DeKAY

Radioactive tracer techniques and statistical methods have been applied to study the uniformity of distribution of several additive phosphorus-32 labeled compounds in a lactose excipient in tablet matrices. Certain physical properties of the additive and excipient powders, the duration of mixing, methods of granulation and drying, solubility of the additive in the granulating agent, additive migration, and other factors, were studied for their effect on the uniformity of additive distribution.

UNIFORMITY and accuracy of drug dosage is considered an essential requirement of most pharmaceutical dosage forms. One of the three major objections which have been made against tablets as a dosage form has been the possibility of nonuniformity of dosage (1, 2). For perhaps this reason Burlinson (3) lists accurate dosage as the most important requirement of a good tablet. Accurate dosage in tablets is controlled by constant tablet weight and a uniform distribution of the additive material throughout the tablet granulation.

With the advent of pharmaceutical drug products of greater potency it is no longer safe to rely on tablet weight variation as the criterion for the uniformity of medicament dosage in tablets. It is obvious that a uniform distribution of potent drugs is necessary in tablet matrices since slight variation in uniformity could produce tablets with more than a slight variation in dosage, particularly when the additional nonuniformity effect of weight variation is considered.

It is essential that the uniformity of drug dispersion in the tablet excipient and the factors affecting this uniformity be known and understood, especially in the preparation of tablets containing only a few milligrams of active ingredient. Existing reports of the factors affecting the uniformity of tablet dosage are few in number and often hypothetical since they completely lack the substantiation of laboratory experimentation.

EXPERIMENTAL

Bloom and Livesey (4) in their distribution studies of additives in animal feeds describe the use of a radioactive isotope as the method of analysis. Necessary modifications of their procedure were adopted using phosphorus-32 as an isotope possessing desirable properties for studying additive distribution in tablet matrices. Phos-

phorus-labeled monobasic potassium phosphate was selected as a water-soluble additive and phosphorus-labeled phosphoric acid was selected as a water and alcohol-soluble additive. The salt was prepared by reaction of $\text{H}_3\text{P}(\text{P}^{32})\text{O}_4$ with anhydrous potassium carbonate, analytical reagent.

A lactose excipient was prepared by drying lactose U. S. P. at 60° , prescreening the dried powder through a number 30 sieve to remove lumps, and finally separating the powder into particle size sieve fractions by a sieve method. Five 5-inch diameter American Society of Testing Materials standard sieves were placed from coarse to fine in a motor driven shaker to separate 100-Gm. portions per separation into sieve fractions. The lactose sieve fractions were analyzed for certain of their properties, including true density and apparent density in order to determine void volume and its significance on uniformity of additive distribution.

Powder mixtures of 0.50% one hundred-mesh labeled phosphate salt in lactose were used in this and subsequent experiments. The specific activity of each phosphorus-labeled monobasic potassium phosphate sample was calculated. Sufficient monobasic potassium phosphate carrier was added to the labeled salt so that the initial corrected activity of a 10.0-mg. sample of lactose powder mixture, containing 0.5% labeled material, would be between 500 and 1,000 counts per minute. Powder mixing was accomplished with a Rampe tumbler mixer,¹ using powder loads of 30 to 1,500 Gm. and mixing jars with a volume greater than three times that of the powder being mixed. Ten or more 10.0-mg. samples were taken from each powder mixture, granulation, or tablet to be studied, weighed on a microbalance, dissolved in water on individual aluminum counting cups, oven dried at 50° , and counted for activity. Each sample activity was corrected for background and resolving time according to the U. S. P. XV method (5). For the measurement of radioactivity a mica window, 24 mg./cm.² Gieger-Mueller tube² was used. The uniformity of activity of the ten or more samples was determined by calculating the per cent coefficient of variation in each instance.

Autoradiographs of granulations and tablets were prepared to provide a gross visual means of

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¹ Rampe Manufacturing Co., Cleveland, Ohio.

² Tracerlab, Inc., 130 High Street, Boston 10, Mass.

studying the distribution of radioactive additive and to verify the method of random sampling and statistical analysis of sample activities for uniformity.

RESULTS AND DISCUSSION

Uniformity Studies of Powder Mixtures.—Mixing studies were conducted by mixing combinations of particle size sieve fractions of additive and excipient for different periods as shown in Table I. Uniformity of distribution of the additive (labeled phosphate) in the powders, granulations, and tablets is reported in the following tables as $V\%$. This is simply the standard deviation/the mean, the expression multiplied by 100 for expression as a per cent. Thus a high $V\%$ means poor distribution of phosphate and a low $V\%$ means a more uniform distribution.

When the particle size of labeled phosphate salt and lactose was simultaneously varied (Table I, powder mixtures 1 through 4), the 200-mesh powder mixture was found to be uniform (powder number 4) and the coarser powder fractions were significantly less uniform (powders number 1, 2, and 3). When the four different particle size fractions of labeled salt were mixed three, fifteen, and thirty minutes with separate 200-mesh portions of excipient powder, the duration of mixing did not significantly affect the uniformity of the coarser three sieve separated fractions (compare powder mixtures 5 through 7, 8-10, and 11-13), but did significantly influence the uniformity of the 200-mesh powders (compare powder mixtures 14-16). The finer particle size distributions of additive require a longer duration of mixing to achieve their optimum uniformity than did the coarser particle size distributions. A fifteen minute tumble mixing was adequate for the

200-mesh powders. For maximum uniformity of additive dispersion in this particular powder mixture of additive and excipient, the results of Table I indicate a 150/200 or 200-mesh additive should be used with a 200-mesh excipient powder.

The void volume (8) of lactose excipient in powders Nos. 17-19 (Table I) was found to be 0.72, 0.84, and 0.86 ml./Gm. The coefficient of variation of these powder mixtures was 59.5, 36.2, and 17.3%. Thus in this case, the differences in distribution between 17 and 19 and 18 and 19 are not due to differences in void volume, but in mesh size of the lactose.

Uniformity Studies of Granulations.—The relative solubility of the granulating agent for the additive and the amount of granulating agent used were studied for their effects on the uniformity of additive distribution in granulations. Sixty-gram powder mixtures containing 0.5% by weight of the labeled phosphate salt in lactose were separately massed in a Mixmaster mixer at speed 2 for three minutes with three different granulating agents, pressed through a number 20 sieve, and oven dried at 50°. The granulating agents used were: water, a solvent for the labeled phosphate additive; dilute alcohol U. S. P., a partial solvent; and alcohol U. S. P., a nonsolvent for the additive. The results of this experiment are shown in Table II.

No significant difference in uniformity of distribution between powder and granulation occurs except in granulation 1 as can be seen from a comparison of $V\%$ values of Table II. Therefore, distribution of additives in granulations can be improved over that of the parent powder when: (a) the additive is sufficiently soluble in the granulating agent and (b) an adequate amount of granulating agent is added (note no change in distribution in

TABLE I.—THE EFFECT OF VARIOUS PARTICLE SIZE DISTRIBUTIONS OF ADDITIVE AND EXCIPIENT ON POWDER UNIFORMITY

Powder Number	KH ₂ P ₂ O ₇ Fraction ^a	Lactose Fraction ^a	Min Mixed	$V\%$ ^b	Significance of Difference at 5% Level ^c
1	80/100	80/100	15	115.6	No—any combination of
2	100/150	100/150	15	135.0	1, 2, and 3
3	150/200	150/200	15	172.6	Yes—1 and 4, 2 and 4, and
4	Through 200	Through 200	15	17.3	3 and 4
5	80/100	Through 200	3	27.1	No—any combination of
6	80/100	Through 200	15	41.8	5, 6, and 7
7	80/100	Through 200	30	42.5	
8	100/150	Through 200	3	25.1	No—any combination of
9	100/150	Through 200	15	22.3	8, 9, and 10
10	100/150	Through 200	30	38.5	
11	150/200	Through 200	3	25.4	No—any combination of
12	150/200	Through 200	15	24.6	11, 12, and 13
13	150/200	Through 200	30	14.9	
14	Through 200	Through 200	3	51.2	No—15 and 16
15	Through 200	Through 200	15	17.3	Yes—14 and 15
16	Through 200	Through 200	30	19.8	14 and 16
17	Through 200	100/150	15	59.5	No—17 and 18
18	Through 200	150/200	15	36.2	Yes—18 and 19
19	Through 200	Through 200	15	17.3	17 and 19

^a The sieve separated powder fractions were designated by ratio expressions in which the numerator indicates the last sieve number the powder passed through and the denominator indicates the sieve on which the powder was retained.

^b $V\%$ is the per cent coefficient of variation (6) $V\% = (s/\bar{X}) 100$, where s is the standard deviation of ten samples and \bar{X} is the mean.

^c To determine the significance of difference between the coefficients of variation of the different powder mixtures the test statistic, $V_1 - V_2/\sigma_{V_1-V_2}$, was compared to the normal curve standard score at the 5% level (7). V_1 and V_2 are the two coefficients of variation being compared and $\sigma_{V_1-V_2} = \sqrt{V_1^2/2n_1 + V_2^2/2n_2}$, where n is the number of samples (ten or more) on which the uniformity of activity of each tablet matrix was determined. This method assumes the ratio $V_1 - V_2/\sigma_{V_1-V_2}$ is normal and is valid when it is predicted before an experiment which a significant difference in the coefficients of variation being compared is significant.

granulation 4). This would imply that when the two conditions above are met, the uniformity of distribution of additive material in a powder mixture may be of secondary importance in obtaining a uniform distribution of additive in a granulation.

Three-hundred and fifty gram fractions of a powder mixture of lactose and labeled phosphates salt were separately massed five minutes in a Mixmaster mixer at speed 2 with water, dilute alcohol U. S. P., and alcohol U. S. P. Each powder mass was divided into seven equal parts, dried by a number of methods, and the uniformity of distribution determined as shown in Table III.

With the exception of granulation 11, the granulations of Table III prepared with dilute alcohol and dried at a temperature exceeding 50° (granulations 10, 12, 13, and 14), were significantly less uniform than the corresponding water granulations (granulations 3, 5, 6, and 7). All of the alcohol granulations

were significantly less uniform than the corresponding water granulations. The granulations prepared with the partial solvent granulating agent of dilute alcohol exhibited the greatest variation in uniformity depending on the method of drying employed, indicating that additive migration may have occurred at the higher drying temperatures. Continually stirring the granulations by hand as they dried under infrared lamps (granulations 7, 14, and 21) produced no significant increase or difference in the uniformity of additive dispersion over the corresponding granulations which were not stirred (granulations 6, 13, and 20).

Autoradiographs were prepared of a number of the granulations shown in Table III (Fig. 1), which varied in uniformity as expressed by the coefficients of variation. The granulations to be autoradiographed were distributed over Scotch brand cellophane tape and exposed to Eastman No-screen

TABLE II.—THE UNIFORMITY OF WATER, DILUTE ALCOHOL, AND ALCOHOL GRANULATIONS OF MONOBASIC POTASSIUM PHOSPHATE AND LACTOSE POWDER MIXTURES

Powder or Granulation ^a		Ml. of Granulating Agent Used Per 100 Gm. of Powder	V% ^b	Significance of Difference Between V% of Granulation and Parent Powder, 5% Level
I.	First Powder Mixture	..	13.5	..
	1. Water Granulation	8.0	6.8	Yes
	2. Dil. Alcohol Granulation	11.2	12.7	No
	3. Alcohol Granulation	25.0	18.6	No
II	Second Powder Mixture	..	16.0	..
	4. Water Granulation	6.5	13.4	No
	5. Dil. Alcohol Granulation	10.0	14.5	No
	6. Alcohol Granulation	27.5	13.9	No

^a The first powder mixture was mixed thirty minutes and the second was mixed ten minutes. Three granulations were made of each powder mixture using granulating agents of water, dilute alcohol U. S. P. and alcohol U. S. P., oven drying the granulations at 50°.

^b The per cent coefficient of variation (Table I, footnote b) in each case was based on a sample size of twelve instead of ten.

TABLE III.—THE EFFECT OF THE DRYING METHOD ON THE UNIFORMITY OF DISTRIBUTION OF LABELED MONOBASIC POTASSIUM PHOSPHATE IN TABLET GRANULATIONS

Granulation Number	Granulating Agent	Method of Drying	V% ^d	Significance of Difference at the 5% Level/
1	Water	Air ^a	13.8	Yes—2 and 7
2	Water	Oven 50°	15.9	No—all other combinations of 1 through 7
3	Water	Oven 75°	10.6	
4	Water	500 Watts I. R. ^b	9.9	
5	Water	375 Watts I. R.	13.7	Yes—8 and 10 Excluding 11; no—all other combinations of 8 through 14
6	Water	250 Watts I. R.	10.7	
7	Water	250 Watts I. R. ^c	8.8	
8	Dil. Alcohol	Air	19.1	
9	Dil. Alcohol	Oven 50°	27.7	No—all combinations of 15 through 21
10	Dil. Alcohol	Oven 75°	44.5	
11	Dil. Alcohol	500 Watts I. R. ^c	14.0	
12	Dil. Alcohol	375 Watts I. R.	35.8	
13	Dil. Alcohol	250 Watts I. R.	31.0	
14	Dil. Alcohol	250 Watts I. R. ^c	24.6	
15	Alcohol	Air	34.0	
16	Alcohol	Oven 50°	36.9	
17	Alcohol	Oven 75°	35.1	
18	Alcohol	500 Watts I. R.	41.6	
19	Alcohol	375 Watts I. R.	25.8	
20	Alcohol	250 Watts I. R.	26.6	
21	Alcohol	250 Watts I. R. ^c	39.3	

^a Air drying was at room temperature, 25°.

^b The infrared lamps were used in pairs in a Fostoria evenray twin lamp holder at a ten-inch lamp to object distance. The maximum temperatures recorded ten inches from the infrared lamps were: 70° with the 250 watt lamps; 80° with the 375 watt lamps; and 95° with the 500 watt lamps.

^c These granulations were constantly stirred by hand as they dried.

^d The per cent coefficient of variation (Table I, footnote b) for granulations 1-7 was based on a sample size of fourteen and for granulations 8-21 on a sample size of ten.

^e The low per cent coefficient of variation of granulation 11 was unexplained. Autoradiographs roughly substantiated this finding.

^f See Table I, footnote c for the test for significance of difference.

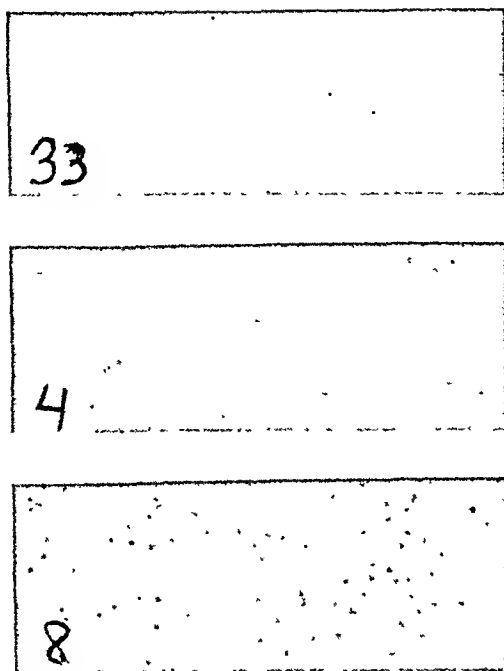


Fig 1.—Granulation Autoradiographs. Granulation number 33 had a coefficient of variation of 4.0%, granulation number 4 (Table III) had a coefficient of variation of 9.9%, and granulation number 8 (Table III) had a coefficient of variation of 19.1%.

X-ray film. Gross visual examination of the granulation autoradiographs indicated complete agreement with the per cent coefficient of variation obtained by the statistical method.

Separate powder mixtures of lactose and labeled phosphate salt were moistened with water and the moistened powders mixed or massed for periods of two and one-half and twenty-five minutes. The

per cent coefficient of variation of the resulting granulations was 18.1 and 20.7% respectively. The difference in the additive uniformity of distribution of the two granulations, depending on the duration of the massing time employed, was not statistically significant at the 5% level.

The water-soluble labeled phosphate additive was dissolved in water, and this solution used as the granulating agent for the lactose excipient. The granulations were air dried at room temperature, oven dried at 50°, and infrared dried at 500 watts, ten inches. The per cent coefficients of variation of these granulations was 7.0, 2.9, and 3.6% respectively. At the 5% level the granulations prepared by dissolving the additive in the granulating agent were significantly more uniform than the corresponding granulations of Table III (granulations 1, 2, and 4) which were prepared by adding the same concentration of granulating agent to the powder mixture of additive and excipient.

The effect on additive uniformity of distribution of the various methods of drying an alcohol and water-soluble additive, phosphoric acid, using the different granulating agents, was studied. The results are shown in Table IV.

When an alcohol solution of the additive was used as the granulating agent (granulations 22–28) infrared drying with the 500-watt lamps (granulation 25) resulted in a granulation significantly less uniform than the other alcohol granulations. Migration of the additive evidently occurred at the temperature of the 500-watt infrared drying method (95°) but was insignificant at the lower temperatures of the other drying methods. Granulation 25 was recounted and the original results were verified. The granulations prepared using a granulating agent of water or dilute alcohol showed no significant migration. Mixing of the dried granulations which had apparently not undergone appreciable migration effects (granulations 22–24 and 26–36) did not significantly increase the uniformity of these granulations. Mixing of dry, sized granulation 25 which had apparently suffered migration effects did significantly increase the uniformity of additive dispersion over that of the same dry, sized granulation before mixing. The per cent coefficient of variation

TABLE IV.—THE EFFECT OF THE DRYING METHOD ON THE UNIFORMITY OF DISTRIBUTION OF AN ALCOHOL AND WATER-SOLUBLE ADDITIVE IN TABLET GRANULATIONS

Granulation Number	Granulating Agent	Method of Drying	1% ^b	Significance of Difference at 5% Level ^c
22	Alcohol	Air	6.6	No, 24 or 28 vs. 25
23	Alcohol	Oven 37°	6.5	
24	Alcohol	Oven 50°	7.3	
25	Alcohol	500 Watts I. R.	13.1	Yes, 22, 23, 26 or 27 vs. 25
26	Alcohol	375 Watts I. R.	6.0	No, other combinations of 22 through 28
27	Alcohol	250 Watts I. R.	6.3	
28	Alcohol	250 Watts I. R. ^a	7.7	
29	Dil. Alcohol	Oven 37°C.	2.7	No significant difference between granulations 29 through 32
30	Dil. Alcohol	Oven 75°C.	2.6	
31	Dil. Alcohol	500 Watts I. R.	1.6	
32	Dil. Alcohol	250 Watts I. R.	1.8	No significant difference between granulations 33 through 36
33	Water	Oven 37°	4.0	
34	Water	Oven 75°	2.8	
35	Water	500 Watts I. R.	2.8	
36	Water	250 Watts I. R.	3.4	

of granulation 25, after the granulation had been tumble mixed for five minutes was 4.8%. Immediately after drying and before mixing the per cent coefficient of variation of the same granulation as shown in Table IV was 13.1.

Some of the granulations in Tables III and IV were separated into fines (pass 60 mesh) and granules (retained on 60 mesh). These were counted (ten 10.0-mg. samples of each fraction) to see if the labeled phosphate additive had a different concentration in the fines and granules. A "t" value was calculated from the corrected average activity of the fine and granule samples and a 1% significance level was used to see if the differences found were significant. On this basis a real difference in concentration of additive was found between fines and granules for the dilute alcohol and alcohol granulations of Tables III and IV studied, the granule fraction in every case being the more active (Nos. 12-18 and 25). No significant difference was found between fine and granule activity of the water granulations studied. Explanation of these findings will require additional work.

Labeled phosphate salt granulations prepared with granulating agents of water, dilute alcohol, and alcohol, and varying considerably in uniformity expressed as the per cent coefficient of variation (6.8 to 22.6%), were lubricated with 1.5% magnesium stearate and compressed into $\frac{3}{8}$ -inch tablets on a Stoke's Eureka hand tablet machine. In every case there was no significant difference in additive uniformity of distribution between ten 10.0-mg. samples from each tablet and the granulation from which the tablet was prepared. Some of the per cent coefficients of variation compared were: 8.0 *vs.* 6.8; 8.2 *vs.* 12.7; 18.9 *vs.* 18.6; 15.3 *vs.* 22.6; and 17.0 *vs.* 13.9. Tablet autoradiographs of $\frac{1}{8}$ -inch diameter tablets prepared on a Carver laboratory press provided substantiation on the basis of gross visual examination of the statistical method of determining tablet uniformity of additive distribution.

SUMMARY

A method employing radioactive tracer techniques for studying medicament distribution in powder mixtures, granulations, and tablets has been developed and found to be a useful tool in such studies.

1. Powders consisting of phosphorus-32 labeled monobasic potassium phosphate as additive and lactose powder U. S. P. as excipient were prepared. The uniformity of distribution of the labeled phosphate was affected by the particle size distribution of additive and excipient and by the mixing time. Best uniformity was obtained with 150/200 or 200 mesh phosphate and 200 mesh lactose tumble mixed for fifteen minutes.

2. The void volume of the lactose excipient had no effect on the uniformity of distribution of the phosphate.

3. The uniformity of drug distribution in a tablet is the same as in the starting powder unless a drug-soluble granulating agent is used. The

best uniformity achieved in this study was $\pm 13.5\%$ in a powder. Thus individual tablets made from this powder will vary as much as 27% in drug content. Using a drug-soluble granulating agent, the granulations varied as little as $\pm 1.6\%$ or no more than 3.2% in the drug content of the resulting tablets. The formulation and the mixing techniques employed in this study were chosen for the purpose of identifying some of the causes of variation of tablet dosage. Consequently the typical size of additive dosage variation encountered in this problem should not be considered as representative of the variation normally encountered in tablet manufacture.

4. When using a granulating agent in which the additive material was soluble, significantly more uniform granulations were usually obtained when the additive was dissolved in the granulating agent than when the granulating agent was added to the powder mixture of additive and excipient.

5. Increasing the massing time beyond the few minutes necessary to uniformly wet the powder mixture with the granulating agent did not significantly increase the uniformity of additive dispersion.

6. The water-soluble, alcohol-insoluble labeled phosphate salt additive when granulated with water or alcohol produced granulations which did not vary significantly in uniformity depending on the drying methods employed (air drying at room temperature, oven drying below 75° or infrared drying below 95°). When this additive in a lactose powder mixture was granulated with dilute alcohol the air dried granulation was significantly more uniform than the granulation oven dried at 75°.

7. The water- and alcohol-soluble labeled phosphoric acid additive, when granulated with water or dilute alcohol produced granulations which did not vary significantly in uniformity due to the drying methods employed. The granulations prepared with an alcohol solution of phosphoric acid additive as the granulating agent did vary significantly in uniformity of additive dispersion depending on the method of drying employed. At the 5% significance level infrared drying at 95° produced a granulation significantly less uniform than infrared drying at 80° or below, oven drying at 37° or below or air drying at room temperature. Stirring the granulations as they dried under infrared lamps, regardless of the granulating agent or additive employed, did not increase the uniformity of additive dispersion over that of granulations not stirred.

8. The concentration of labeled phosphate

additive in alcohol and dilute alcohol granulations was different between material retained on a 60-mesh screen (granules) and material passing a 60-mesh screen (fines). This was not the case when water was the granulating agent.

9. The uniformity of additive dispersion within compressed tablets was found to be statistically identical to the degree of uniformity of additive distribution of the granulations from which the tablets were made.

REFERENCES

- (1) Kuever, R. A., *THIS JOURNAL*, 17, 365 (1928)
- (2) Husa, W. J., *ibid.*, 17, 38 (1928)
- (3) Burlinson, H., *J. Pharm. J.*, 164, 437 (1950)
- (4) Bloom, C., and Livesay, E. F., *Mfg. Chemist*, 24, 371 (1953)
- (5) United States Pharmacopoeial Convention, "The Pharmacopoeia of the United States of America," 1955, Rev. 15, Mack Publishing Co., Easton, Pa., p. 822
- (6) Ostle, B., "Statistics in Research," The Iowa State College Press, Ames, Iowa, 1954, pp. 25, 51, 91, and 98
- (7) Kendall, M. G., "Advance Theory of Statistics," C. Griffin and Co., London, 1945, v. 1, p. 209
- (8) Dallavalle, J. M., "Micromeritics: the Technology of Fine Powders," 2nd ed., Pitman Publishing Corp., New York, 1948, pp. 68, 96, 113, 123, and 131

Potentiometric and Amperometric Titrations of Ascorbic Acid*

By S. M. DESHPANDE and R. NATARAJAN

Potentiometry and amperometry using a dropping mercury electrode have been tried and found suitable for the estimation of ascorbic acid by titration with potassium permanganate. It has been confirmed that there is a necessity for the addition of potassium iodide to get the end point at the dehydroascorbic acid stage. Quantities less than 1 mg. of ascorbic acid could be estimated potentiometrically within ± 1.5 per cent deviation. The possibility by amperometry, of other permanganometric titrations using potassium iodide as indicator, has been suggested.

THE 2,6-DICHLOROPHENOL-INDOPHENOL METHOD (1) for the estimation of ascorbic acid has certain inherent defects, such as the instability of the dye solution necessitating standardization often, and the transient nature of the end point which introduces considerable difficulties. In an attempt to find a suitable alternate method, the permanganometric method of Murti and Viswanadham (2) was considered. They have reported that the end point in lower concentrations of ascorbic acid was achieved at equivalent proportions only by use of starch-potassium iodide as an indicator; furthermore, a deviation of $\pm 2.64\%$ in titrations using 0.001 *N* permanganate for amounts less than 1 mg. of ascorbic acid, has been reported. Here, the permanganate is expected to first liberate iodine from potassium iodide, which in turn reacts with ascorbic acid to give dehydroascorbic acid. It was therefore thought of confirming the necessity for the addition of potassium iodide, by other methods. Attempt has also been made to see whether the error could be minimized by any other suitable method.

EXPERIMENTAL

Chemicals of AR grade and ascorbic acid (E. Merck) which was standardized by iodimetric method

(3) were used in these experiments. Further, the use of conductivity water as recommended by Murti and Viswanadham for the permanganate method was considered not practicable for routine analysis and therefore ordinary distilled water was employed. Ascorbic acid solutions were prepared as and when required, by taking requisite amount in 100 ml of water containing 4 ml of 2 *N* sulfuric acid as stabilizing agent; such solutions were found to be stable for about two hours at room temperature ($25 \pm 2^\circ$).

Potentiometry.—Pye's "S" model which gives an accuracy of 1 mv. and a constant stirrer assembly for mixing the solution were used. One ml. of ascorbic acid solution was taken in a titration vessel of 100 ml capacity, with 2 ml of 2 *N* sulfuric acid and 1 ml. of 0.5% potassium iodide solution along with 50 ml. of water. This solution was titrated with 0.001 *N* permanganate (except where indicated, see Table I) using a platinum wire electrode as the indicator and saturated calomel electrode as the reference electrode. The E. M. F. of the solution after one minute of each addition of permanganate was noted by the null deflection method; the titration was continued up to about 2 ml. excess addition of permanganate after the end point, as usual. The equivalence point for each titration was determined by plotting $\Delta E/\Delta V$ against volume of permanganate added; a typical curve obtained is given in Fig. 1.

Amperometry.—The polarographic equipment used for this purpose was made by Messrs. Adept Laboratories, Poona, India; this was used in conjunction with a spot type suspension coil galvanometer and a dropping mercury electrode.

In the polarographic cell, a solution containing 1 ml. of a solution of ascorbic acid, 2 ml. of 2 *N* sulfuric acid, 1 ml. of 0.5% potassium iodide, and 40 ml. of water was taken. The dropping mercury

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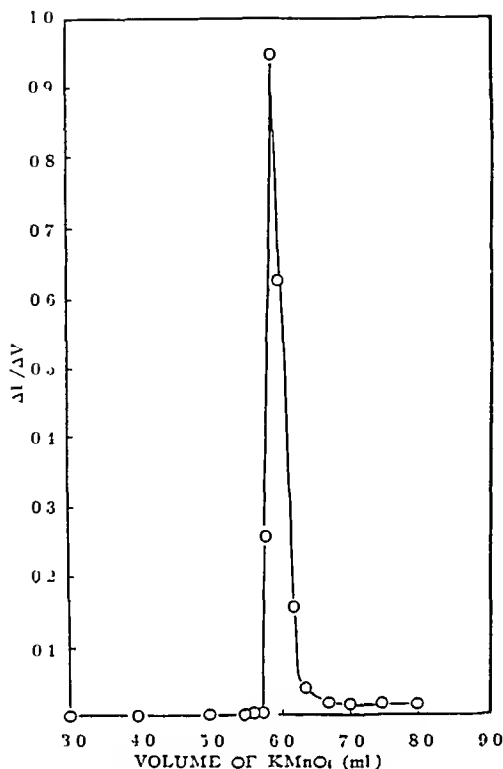


Fig 1—Potentiometric determination of ascorbic acid (0.5 mg).

electrode was dipped into the solution so as to be 1 cm below the surface of the liquid, and the mercury level head was adjusted to give a drop time of 3.33 seconds. A constant potential of -0.1 volt vs S.C.E. was applied and the titration with permanganate ($0.01 N$) was carried out noting the deflection of the galvanometer after each addition. The galvanometer deflection was plotted against volume of permanganate added, to get the end point, a typical curve obtained is given in Fig 2.

RESULTS AND DISCUSSION

Potentiometry.—When ascorbic acid was titrated against potassium permanganate, a sharp end point could not be obtained, the galvanometer deflections were not steady, but showed a tendency to fall back to the original level in about a minute. This is because the reaction does not stop at the dehydroascorbic acid stage as reported by Herbert, *et al* (4). However, the addition of potassium iodide above 1 ml of 0.5% brought about a sharp end point at the dehydroascorbic acid stage indicating that the permanganate liberates iodine from potassium iodide after the equivalence point in preference to the further oxidation of dehydroascorbic acid. Or, perhaps from the beginning, permanganate liberates iodine from potassium iodide which, in turn, oxidizes the whole ascorbic acid, and finally the free iodine produces the change of potential. In any case, the reaction has been found suitable for potentiometric determination of small quantities of ascorbic acid by titration with even $0.001 N$ permanganate in presence of potassium iodide.

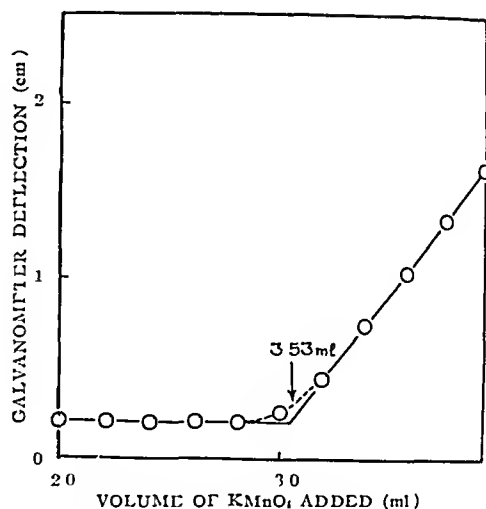


Fig 2—Amperometric determination of ascorbic acid (3.0 mg).

ascorbic acid by titration with even $0.001 N$ permanganate in presence of potassium iodide.

The presence of potassium iodide in the range of 1 ml of 0.5 to 8% solution gave consistent values for the end point. Similarly, sulfuric acid concentration could be altered between 1 and 5 ml of 2 N strength without affecting the results. Therefore, for convenience, 1 ml of 0.5% potassium iodide and 2 ml of 2 N sulfuric acid as recommended by Murthy and Viswanadham were employed and the results thus obtained for various concentrations of ascorbic acid are given in Table I. In addition, a comparison has been made of the results obtained by potentiometry with the values obtained by direct titration using 1 ml of 0.5% starch as indicator.

TABLE I—ASSAY OF ASCORBIC ACID

Ascorbic Acid Taken, mg	By Potentiometry—		By Direct Titration Using Starch as Indicator	
	Ascorbic Acid Found, mg	Percentage Deviation	Ascorbic Acid Found, mg	Percentage Deviation
0.30	0.302	+0.67	0.289	-3.60
0.50	0.495	-1.00	0.503	+0.60
0.80	0.789	-1.37	0.813	+1.63
1.00	0.990	-1.00	0.996	-0.40
3.00 ^a	3.018	+0.60	3.018	+0.60
4.00 ^a	3.983	-0.43	4.065	+1.60
6.00 ^a	6.037	+0.61	6.076	+1.27

^a $0.001 N$ permanganate was used for the others $0.001 N$ permanganate.

Amperometry.—Ascorbic acid in sulfuric acid medium does not give any polarographic wave. Potassium permanganate is reported to give no polarographic wave suitable for analytical purpose (5). Therefore, the amperometric titration of ascorbic acid with permanganate directly is not possible. The permanganometric method using potassium iodide can, however, be made the basis of an amperometric method.

The deviation obtained in potentiometry in the

region of lower concentrations of ascorbic acid is of the order of $\pm 1.5\%$

From preliminary experiments it was found that consistent values were obtainable at an applied potential of -0.1 volt vs. S. C. E. in this method. Accordingly, various amounts of ascorbic acid were titrated, and the results are given in Table II, along with the values obtained by direct titration.

TABLE II.—AMPEROMETRIC DETERMINATION OF ASCORBIC ACID

Ascorbic Acid Taken, mg	By Amperometry—		By Direct Titration Using Starch as Indicator	
	Ascorbic Acid Found, mg.	Percentage Deviation	Ascorbic Acid Found, mg.	Percentage Deviation
1.0	0.995	-0.50	1.012	+1.2
2.0	2.007	+0.35	2.024	+1.2
3.0	3.002	+0.06	3.045	+1.5
4.0	3.973	-0.67	4.024	+0.6

Strength of pot. permanganate, 0.01 *N*. Applied potential, -0.1 volt vs S. C. E. Galvanometer shunt, $1/2$

Results obtained by this method, however, showed that the amperometric method is not sensitive enough with 0.001 *N* permanganate. It is

also evident that whenever permanganate is to be used in amperometric titrations, potassium iodide may serve as an indicator.

SUMMARY

The permanganometric method of titrating ascorbic acid in presence of potassium iodide and sulfuric acid has been tried by potentiometry and amperometry. Here potassium iodide acts as an indicator. The potentiometric method gives an error of $\pm 1.5\%$ for ascorbic acid less than 1 mg., whereas the amperometric method is not sensitive enough below 1 mg. of ascorbic acid.

It should be possible to extend this method for other permanganate titrations which are not normally possible by amperometry.

REFERENCES

- 1 Bessey, O. A., *J. Biol. Chem.*, 126, 771 (1938)
- 2 Murti, K. S., and Viswanadham, C. R., *Proc. Nat. Inst. Sci. India*, 10, 217 (1948), *Chem. Abstr.*, 42, 8833 (1948)
- 3 "British Pharmacopoeia," 1953
- 4 Herbert, R. W., Hirst, E. L., Percival, E. G. V., Reynolds, R. J. W., and Smyth, F., *J. Chem. Soc.*, 1270 (1933)
- 5 Stackelberg, M., v., Klunger, P., Koch, W., and Krath, E., *Tech. Mitt. Krupp A. Forschungsber.*, 2, 59 (1939)

Evaluation of Oral Antiseptic Products on Buccal Epithelial Tissue*

By LEONARD J. VINSON and ALLAN G. BENNETT

Two *in vivo* methods for evaluating antiseptic properties of oral products such as mouthwashes and dentifrices are described. Buccal tissue scrapings removed by curettage serve as the test substrate in estimating oral bacterial count (Buccal Tissue Count Test) and in measuring the degree of affinity of the antiseptic to mouth tissue after brushing with a dentifrice or swishing with a mouthwash (Buccal Epithelial Substantivity Test). The test cultures employed were *M. pyogenes* var. *aureus* and *L. casei*. The buccal tissue shows inhibitory effect against oral bacteria lasting up to four hours after one treatment of a hexachlorophene-containing mouthwash or dentifrice.

DESPITE the large number of tests reported in the literature a reliable *in vivo* method is still needed for evaluating antiseptic oral products with particular reference to dentifrices and mouthwashes. Many factors appear to influence the type and numbers of the oral flora in the mouth. Saliva counts, for example, fluctuate widely during the course of a day being influenced by diet, by changes in salivation rate, and by environmental factors. Thus, counts made on stimulated or unstimulated saliva are poorly reproducible and do not serve as a good basis for evaluating oral antiseptics (1). The rinse technique as described by Ostrolenk and co-workers

(2) and Watson and Reddish (3) is an improvement, but the shortcomings of the saliva count method remain.

The swab technique as described by Bloomfield (4) and many modifications thereof employ oral surfaces such as teeth, tongue, buccal epithelium, and palate. Again, the wide variability in counts encountered makes it a poor choice for evaluating oral antiseptic agents.

As indicated by Ostrolenk and co-workers (2) the evaluation of the effectiveness of oral antiseptics must primarily rely upon securing representative samples of the oral microbial population before and after use of the product. It is the purpose of this report to describe a new method for evaluating oral antiseptic agents using buccal

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epithelial scrapings from the mouth. These are examined for the presence of adsorbed antiseptic agent, and bacterial counts are obtained.

METHODS

Buccal Tissue Count Test. Buccal epithelial scrapings are readily obtained from a subject's mouth by means of a curette.¹ This is done simply and easily by the subject himself, who gently scrapes the inside of either cheek using several strokes until the cuplike receiver of the curette is filled with a mixture of mucus-epithelial detritus. With a little experience the amount removed is readily standardized as reflected in the reproducible counts obtained. The tissue is transferred from the curette by agitation into 10 ml sterile 0.1% peptone water contained in a screw-capped test tube. The contents are shaken thoroughly, diluted further when necessary, and 1 ml. aliquots in duplicate plated directly into Blood Agar Base (Difco). Counts are made after forty-eight hours incubation at 37°.

Buccal Epithelial Substantivity Test.—Buccal epithelial scrapings are removed from the mouth as described previously. The tissue is scooped out of the curette with the tapered end of a metal spatula and transferred to the center of the filter paper disk.² The disks with the flattened tissue are allowed to dry for one hour at 55° and then placed tissue side down on seeded agar plates. In the case of the test organism, *M. pyogenes* var. *aureus*,³ Nutrient Agar is employed and for *Lactobacillus casei*⁴ the medium is Tryptone Glucose Extract (TGE) Agar with 2% added yeast extract. A number of disks can be placed peripherally (3/4 inch from the edge) on the agar surface. Additional disks can be situated in the center area. Thus, buccal tissue specimens from one or more subjects can be conveniently placed on the same seeded agar plate.

The plates are incubated overnight at 37°. The disks are removed and the zones of tissue contact with agar are examined microscopically for evidence of bacterial colonies, foaming at the surface. This is accomplished by bringing into sharp resolution the imprint of the disk and epithelial tissue on agar. By employing a reticule (Howard micrometer disk) the colonies in four squares picked at random in the central contact region are counted and a similar count is made in an adjacent agar area not in contact

with the buccal scrapings (control counts). The per cent reduction in count of area in contact with the buccal tissue is determined on the basis of the control count.

Buccal Tissue Count Test Results.—The antiseptic action of dentifrices and mouthwashes on oral flora is evaluated by running buccal tissue counts before and after use of the products.

The reproducibility of counts was determined on replicate samples of buccal tissue from each of eight subjects. Results are given in Table I.

The replicate series yield counts showing good reproducibility. Fluctuations do occur between the average value of a series and that obtained at a different time during the day on the same subject. The better reproducibility in average counts made on buccal scrapings is due to the use of replicate samples. It is not possible to obtain replicates of saliva at one sampling period, since successive saliva samples would be representative of a changing oral condition as fresh saliva is secreted to replace that taken in sampling. With the buccal scrapings technique, four essentially simultaneous samples can be taken without disturbing the salivation rate. Representative buccal count data obtained on three subjects after single brushings with plain and antiseptic dentifrices, and with water are shown in Table II. A decided drop in buccal count occurred after brushing with the antiseptic dentifrice. The individual final counts in every instance fell considerably below even the lowest initial value. This was not observed when the subjects brushed with an ordinary dentifrice, the reductions in count being only moderate. Subjects on the water control showed no reductions in count.

The apparent increase in buccal counts after a water wash is not real and is attributable to the fact that a more diligent scraping over a wider area of the buccal tissue is required to obtain a full sample in the curette. The fact that this increase in count does not occur after use of a dentifrice or mouthwash is explained by the partial destruction of buccal bacteria on immediate contact with the preparation.

Dentifrices and mouthwashes were evaluated for antiseptic activity employing the buccal tissue count test. Included in this series were a toothpaste and a mouthwash containing hexachlorophene, at 0.05% and 0.02% levels, respectively. Twenty-three to thirty subjects were employed for each product and a water control. The procedure was standardized as follows: The subject brushed with the

TABLE I.—REPRODUCIBILITY OF BACTERIAL COUNTS OF REPLICATE BUCCAL TISSUE SCRAPINGS*

Buccal tissue replicate	Subjects							
	1	2	3	4	5	6	7	8
a	52,160	48,420	132,480	123,840	2,740	53,020	16,000	8,640
b	46,450	56,000	115,200	103,680	5,120	89,280	15,630	16,000
c	39,680	66,780	69,120	123,840	2,880	70,080	16,960	7,320
d	60,380	57,820	135,360	98,000	5,120	74,880	18,240	12,800
Mean	49,668	57,255	113,040	112,340	3,965	71,815	16,708	11,190

* The scrapings collected in a curette are obtained after several contact strokes on the inside of the mouth (buccal tissue). The area samples represent a small fraction of the mouth tissue.

¹ Mayhoefer chalazion curette, No. 3.
² Whatman #2, 12.7 mm diameter.
³ Twenty-four hour culture in Nutrient Broth (Difco)—1% inoculum.
⁴ Twenty-four hour culture in Microinoculum Broth (Difco)—1% inoculum.

dentifrice or swished with the mouthwash once in the morning for a period of one minute followed by two five-second water rinses. Before and immediately after the oral treatment buccal scrapings were taken for bacterial counts in the prescribed manner.

Two hours later repeat buccal tissue scrapings were removed for analysis. Average per cent reductions in bacterial counts are given in Table III.

count rose after two hours to a value actually higher than the initial. The water control was without effect in the buccal tissue count test.

TABLE II.—EFFECT OF SINGLE BRUSHINGS WITH AN ANTISEPTIC DENTIFRICE, PLAIN DENTIFRICE, AND WATER ON BUCCAL TISSUE COUNTS (4 REPLICATES)

Subject ^a	Water		Plain Dentifrice		Antiseptic Dentifrice	
	Before	After	Before	After	Before	After
1	2,740	6,400	9,280	3,140	10,160	460
	5,120	8,960	11,520	1,500	11,280	1,620
	2,880	17,280	11,200	1,650	8,480	210
	5,120	6,400	11,200	9,920	9,280	120
	Mean	3,965	9,760	10,725	4,053	9,800
2	53,020	66,240	8,640	1,030	34,130	280
	89,280	86,400	7,360	2,150	33,760	340
	70,080	67,200	8,320	7,360	30,390	360
	74,880	77,760	8,320	7,040	9,920	430
	Mean	71,815	74,400	8,160	4,395	29,278
3	8,640	30,080	13,760	2,940	33,240	1,980
	16,000	23,360	20,480	5,350	39,620	920
	7,320	26,880	15,360	4,480	39,960	1,060
	12,800	27,520	23,680	5,440	43,050	1,020
	Mean	11,190	26,960	18,320	4,553	38,968

^a Brushings with dentifrices and water made on different days.

TABLE III.—PER CENT REDUCTIONS IN BACTERIAL COUNTS OF BUCCAL TISSUE SCRAPINGS FOLLOWING A SINGLE ORAL TREATMENT^a WITH COMMERCIAL DENTIFRICES AND MOUTHWASHES

	No of Subjects	Antiseptic Component	Antibacterial Effectiveness			
			Immediately After Av. % Reduction	Reversals, ^b %	2 Hours Later Av. % Reduction	Reversals, %
Dentifrice:						
A	27	0.05% Hexachlorophene	94	0	45	11
B	26		49	12	3	54
C	28		35	25	-20	60
D	28		52	10	-36	78
E	30		37	27	-14	53
Mouthwash:						
F	28	Thymol; boric and benzoic acid; alcohol	61	14	-24	64
G	23	0.02% Hexachlorophene; alcohol	95	0	30	17
Water	26		-22	77	-26	69

^a The subject brushed with a dentifrice for one minute followed by two five-second rinses with water. With the undiluted mouthwash or water control, the subject swished 10-20 ml sample for 1 minute, followed by two five-second rinses with water.

^b A reversal refers to a buccal tissue count higher after treatment than before.

From the results, it is evident that the dentifrices and mouthwashes act to give an immediate reduction in buccal tissue count. Dentifrice A with 0.05% hexachlorophene gave an average reduction in bacterial count of 94%. This was significantly better than the other dentifrices. The reduction in buccal tissue count effected by Dentifrice A was still evident two hours after the single treatment. The other products did not show any residual effect. The outstanding action of Dentifrice A is also apparent in comparing the reversals (increase in count) for the different preparations. With the dentifrice containing hexachlorophene, there were no reversals immediately after treatment and a significantly lower number of reversals two hours later. Mouthwash G with 0.02% hexachlorophene gave an immediate reduction in buccal tissue count of 95% and the count remained depressed for at least two hours. Mouthwash F with thymol and other ingredients showed a 61% immediate reduction but the average

Buccal Epithelial Substantivity Test Results.—

The ability of dentifrices and mouthwashes to show a residual antiseptic effect on oral flora after brushing or swishing was investigated employing the Buccal Epithelial Substantivity Test described previously. Buccal tissue scrapings were removed from subjects' mouths in the designated manner prior to a single treatment^b with an oral product. Immediately after use of the product, and one, two, and four hours later, additional samples were taken. Duplicate samples of buccal tissue scrapings for each period were evaluated for their inhibitory effect, one against *M. pyogenes* var. *aureus* and the other against *L. casei*. Per cent reductions in bacterial counts are recorded in Table IV.

The results give convincing evidence of the affinity of hexachlorophene to buccal tissue of subjects' mouths treated with Dentifrice A containing 0.05%

^b One minute brushing or swishing followed by two five-second rinses.

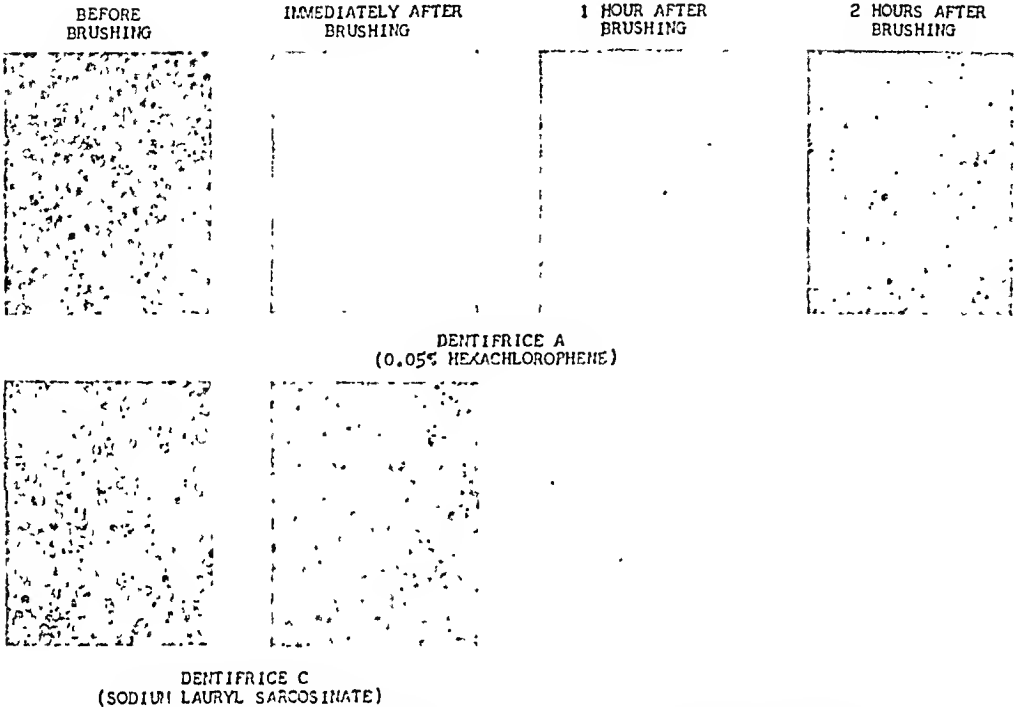


Fig 1 Photomicrographs (175 X) of contact areas of buccal tissue to agar showing the sustained inhibitory effect of dentifrice A against *Lactobacillus casei*. Dentifrice C is without effect.

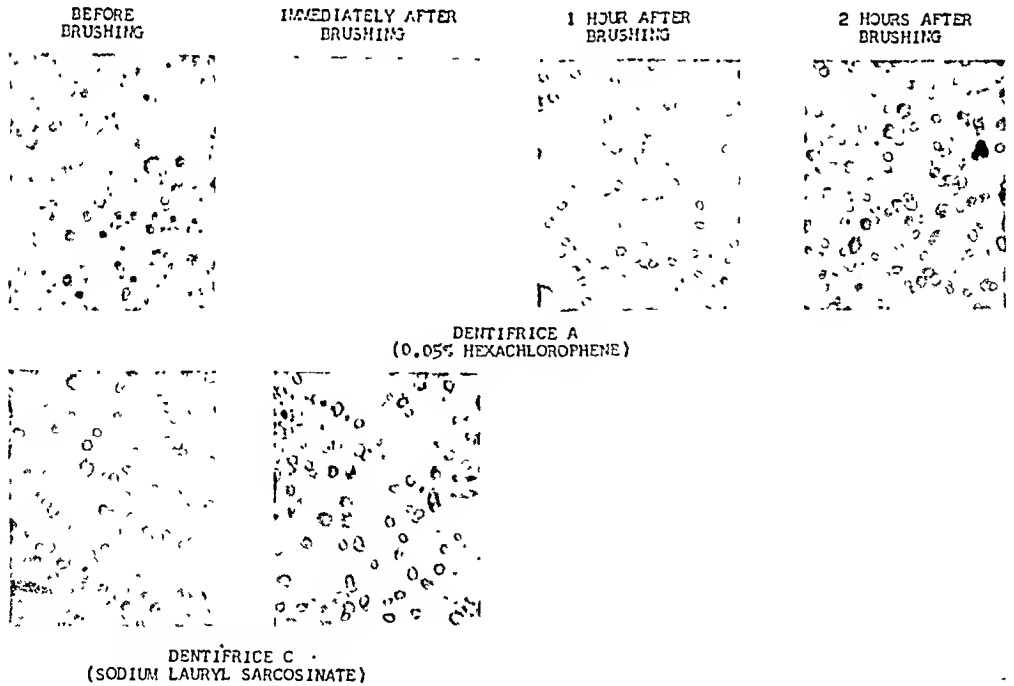


Fig 2 —Photomicrographs (175 X) of contact areas of buccal tissue to agar showing the sustained inhibitory effect of dentifrice A against *M. pyogenes* var. *aureus*. Dentifrice C is without effect.

TABLE IV.—BUCCAL EPITHELIAL SUBSTANTIVITY DATA FOR DENTIFRICES AND MOUTHWASHES

		Reduction in Bacterial Counts of Contact Area, %									
Antiseptic Ingredient		Before Treatment		Immediately After		1 Hour Later		2 Hours Later		4 Hours Later	
		M ^a	L ^b	M	L	M	L	M	L	M	L
Dentifrice:											
A	0.05% G-11	<10	<10	100	100	73	74	55	46	<10	32
B		<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
C		<10	<10	27	10	<10	<10	<10	<10	<10	<10
D		<10	<10	<10	<10	15	<10	<10	<10	<10	<10
Mouthwash:											
F	Thymol; boric and benzoic acid; alcohol	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
G	0.02% G-11; alcohol	<10	<10	100	100	75	100	46	77	21	23
Saline	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

^a M denotes *Micrococcus pyogenes* var. *aureus*. ^b L denotes *Lactobacillus casei*.

hexachlorophene or Mouthwash G with 0.02% hexachlorophene. Immediately after brushing with the paste or swishing with mouthwash the buccal tissue develops strong antibacterial activity, as indicated by the complete inhibition of bacterial growth. The inhibitory nature of the buccal tissue persists at least up to four hours after the treatment. Ordinary dentifrices and Mouthwash F show little or no effect in developing an antibacterial mantle on buccal tissue. Saline shows no effect in this test.

Photomicrographs of seeded agar surfaces exposed to buccal tissue scrapings are shown in Figs. 1 and 2. The areas shown contrast the action of the hexachlorophene dentifrice which imparts strong antibacterial activity, with that of an ordinary dentifrice showing no activity.

DISCUSSION

Two reliable methods employing buccal tissue for evaluating the antiseptic properties of oral products like dentifrices and mouthwashes are described. Wide fluctuations in counts are not the rule as observed in the older methods employing saliva as the substrate.

The reduction in buccal tissue counts is considered a sound measure of the effectiveness of oral antiseptic products since the tissue is constantly exposed to bacteria-rich saliva. Buccal tissue counts, in a sense, reflect the oral cavity environment with respect to type and number of bacteria. It is, therefore, noteworthy that the buccal count remains relatively low in the presence of a tissue-substantive antiseptic like hexachlorophene in a mouthwash or dentifrice.

Hexachlorophene is known to be a potent bacteriostatic agent at very low concentrations. Ennever and co-workers (5) reported that growth of oral bacteria (pooled human saliva) and resultant acid production were completely inhibited in a dextrose-peptone medium containing as low as 0.125 p. p. m. hexachlorophene.

Florestano and co-workers reported (6) that organisms such as streptococci, staphylococci, pneumococci, lactobacilli, fusiform bacillus, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Borrelia vincenti*, and other spirochetes are constantly found in the mouth both in health and disease. They point out that the mouth tissue cannot be disinfected and the only practical approach to control potentially harmful oral microorganisms is to keep down their numbers.

The action of oral products in controlling bacterial growth in the mouth is readily assessed by the buccal tissue count test. The ability of an antiseptic in an oral product to cling to the mouth tissues and to continue to act for a sustained period is readily demonstrated in the buccal epithelial substantivity test. In the cases of a hexachlorophene-containing dentifrice or mouthwash, the buccal epithelial substantivity test data show a persistence of the antiseptic on buccal tissue at least up to four hours after a single oral treatment.

The buccal epithelial substantivity test can be modified by employing other test organisms to determine the activity of the oral products against a wide spectrum of bacteria. For example, in addition to testing *M. pyogenes* var. *aureus* and *L. casei*, other organisms such as those noted by Florestano (6) and even a heterogeneous oral flora culture from saliva can be used. Antibacterial activity for mouth tissue in the buccal epithelial substantivity test is a strong indication that bacteria on oral tissues *in vivo* are unable to multiply.

Substrates other than buccal tissue scrapings have also been used in assessing substantivity of antiseptics to oral surfaces. For example, tooth plaques can be removed from the mouth and shown to have an inhibitory effect against bacteria following oral treatment with the hexachlorophene-containing mouthwash or dentifrice.

The buccal tissue count test employing differential media is applicable in evaluating the effectiveness of antiseptic oral products with respect to the different bacterial species that comprise oral flora. For example, by plating out buccal scrapings samples into blood agar, the hemolytic streptococci and micrococci can be detected and counted. Hexachlorophene-containing dentifrice and mouthwash were found to be effective in reducing the buccal tissue counts of these hemolytic cocci. The reduction persisted for several hours.

REFERENCES

- (1) Hadley, F. P., *J. Dental Research*, 13, 415(1933).
- (2) Ostrolenk, M., Meyers, C. E., and Mollahi, M., *THIS JOURNAL*, 41, 89(1952).
- (3) Watson, N. E., and Reddish, G. F., *ibid.*, 45, 678 (1956).
- (4) Bloomfield, A. L., *Bull. Johns Hopkins Hosp.*, 33, 145(1922).
- (5) Ennever, J., Robinson, H. B. G., and Kitchin, P. C., *J. Dental Research*, 32, 61(1953).
- (6) Florestano, H. J., Bahler, M. E., and Richards, A. B., *THIS JOURNAL*, 45, 13(1956).

An Evaluation of the Pharmacologic Activity of a New Series of Chalcone Derivatives*

By G. VICTOR ROSSI and ELIAS W. PACKMAN

A series of fourteen new chalcone derivatives, synthesized as potential antagonists of norepinephrine biogenesis, were evaluated with regard to their hypotensive potency in the normotensive dog. The compound 2-(2-dimethylaminoethoxy) chalcone citrate, selected as the most active member of this group, was subjected to further study including determination of depressor effect in the hypertensive rat, acute toxicity, influence on epinephrine toxicity, and modification of barbiturate induced "sleeping time." This series of chalcones was also screened for antihistaminic and antispasmodic activity on isolated guinea pig ileum, and the three most effective compounds in this respect were examined for their ability to inhibit the histamine depressor response in the cat. The chalcones investigated in this study compared unfavorably with available therapeutic agents in regard to specificity, potency, and duration of action. Recommendation is made for synthesis of additional chalcones with consideration given to the activities reported.

CLARK (1) REPORTED in 1953 that certain hydroxychalcones inhibit 3,4-dihydroxyphenylalanine (dopa) decarboxylase. Presuming that active chalcones could be synthesized, this finding suggested the possibility of blocking the biogenesis of norepinephrine, the adrenergic neurohumoral transmitter. Such compounds might reasonably be expected to possess hypotensive activity.

Hydroxychalcones have been found to interfere with the activity of various enzyme systems. Beiler (2) has shown that these compounds inhibit the activity of choline acetylase. Certain substituted chalcones, notably 3,3',4,4'-tetrahydroxychalcones, were found by Beiler and Martin to function as effective inhibitors of rat liver xanthine oxidase *in vitro* (3) and *in vivo* (4). Hydroxychalcones were reported by Bartlett (5) to inhibit succinoxidase activity, probably due to the formation of quinones which react with essential sulfhydryl groups of the enzyme.

On the basis of Clark's observations (1), Packman and Rubin (6) prepared a series of chalcone derivatives as potential antagonists of norepinephrine biosynthesis. This report is primarily concerned with an evaluation of the blood pressure reducing activity of these compounds. However, certain of the structural features of these chalcones also warranted investigation of their antihistaminic activity. Notably, the groups joined by ether linkage to the chalcone nucleus are disubstituted alkylamino moieties, forming a structural class to which many potent antihistamines belong. The terminal nitrogen is tertiary, with some of the compounds possessing the N-dimethyl group generally found to be optimal for antihistaminic activity.

EXPERIMENTAL

Of the original series of compounds synthesized by Packman and Rubin (6), those listed in Table I were available for pharmacologic evaluation.¹ Henceforth in this report the compounds will be referred to by the code letter assigned for the investigation.

Hypotensive Activity in Normotensive Dogs.—Fourteen chalcone derivatives were examined for their ability to lower blood pressure and reduce the pressor response to epinephrine and occlusion of the carotid arteries in healthy, normotensive, vagally denervated mongrel dogs, anesthetized with 35 mg./Kg. of pentobarbital sodium injected intravenously. Blood pressure was recorded directly from a femoral artery. In this study the chalcones were injected intravenously (femoral vein) in geometrically progressing doses beginning with 1 mg./Kg. Succeeding doses were injected after the blood pressure returned to pretreatment level or after an interval of twenty minutes if the preceding dose had no significant effect. This procedure continued until the death of the animal occurred. Epinephrine hydrochloride (0.25 to 0.5 ml. of a 1:20,000 solution, depending upon the sensitivity of the animal) was injected intravenously ten minutes after administration of each dose of the chalcone. Five minutes after the injection of epinephrine, the carotid sinus reflex was evoked by clamping both common carotid arteries for an interval of thirty seconds. The pressor responses following each procedure were compared with a series of uniform responses elicited prior to administration of the first dose of chalcone. The data obtained in this study are summarized in Table II.

With the exception of compound A, none of the chalcones examined produced a sustained (sixty minutes or more) reduction of blood pressure in sublethal concentrations. None of the animals survived the 32 mg./Kg. intravenous dose (representing a cumulative concentration of 63 mg./Kg.); in many cases death occurred rapidly after administration of the 16 mg./Kg. dose. Immediate post-mortem

* Received from the LaWall Memorial Laboratory of Pharmacology and Biochemistry, Philadelphia College of Pharmacy and Science, Philadelphia 4, Pa.

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¹ The chalcones employed in this study were generously supplied by Dr. Nathan Rubin, Department of Chemistry, Philadelphia College of Pharmacy and Science, and Dr. Albert Packman, Department of Organic Chemistry, National Drug Company Research Laboratories, Philadelphia, Pa.

TABLE I.—A NEW SERIES OF CHALCONE DERIVATIVES^a

<div style="text-align: center;"> $\begin{array}{c} \text{CH}=\text{CH}-\text{C}=\text{O} \\ \qquad \qquad \\ \text{C}_6\text{H}_4 \qquad \text{C}_6\text{H}_4 \\ \text{4} \qquad \qquad \text{4}' \end{array}$ <p>Chalcone</p> </div>			
Code Letter	Group Substituted on Chalcone	Position on Chalcone	Anion
A	(CH ₃) ₂ NCH ₂ CH ₂ O—	2	Citrate
C	(C ₂ H ₅) ₂ NCH ₂ CH ₂ O—	2	Citrate
D	(C ₂ H ₅) ₂ NCH ₂ CH ₂ CH ₂ O—	4	HCl
E	(CH ₃) ₂ NCH ₂ CH ₂ O—	4	Citrate
F	(CH ₃) ₂ NCH ₂ CH ₂ CH ₂ O—	4	HCl
G	(C ₂ H ₅) ₂ NCH ₂ CH ₂ O—	4'	HCl
H	(CH ₃) ₂ NCH ₂ CH ₂ CH ₂ O—	4'	HCl
I	(C ₂ H ₅) ₂ NCH ₂ CH ₂ O—	2'	Citrate
J	(CH ₃) ₂ NCH ₂ CH ₂ O—	4	HCl
K	(CH ₃) ₂ NCH ₂ CH ₂ O—	4'	HCl
M	(CH ₃ —CH—) ₂ NCH ₂ CH ₂ O—	4	HCl
<div style="text-align: center;"> $\begin{array}{c} \text{CH}_3 \\ \\ \text{(C}_2\text{H}_5)_2\text{NCH}_2\text{CH}_2\text{O—} \end{array}$ <p>Additional Compounds</p> </div>			
<div style="text-align: center;"> $\begin{array}{c} \beta \qquad \alpha \\ \text{CH}=\text{CH}-\text{C}=\text{O} \\ \qquad \qquad \\ \text{C}_6\text{H}_4 \qquad \text{S} \\ \text{4} \end{array}$ <p>Alpha-(2-thenoyl)-beta-phenylethylene</p> </div>			
Code Letter	Group Substituted on Benzene Ring	Position on Benzene Ring	Anion
B	(CH ₃) ₂ NCH ₂ CH ₂ O—	4	HCl
L	(C ₂ H ₅) ₂ NCH ₂ CH ₂ O—	4	Citrate

^a The method of numbering the substituted chalcones is based on the system used in *Chemical Abstracts*.

examination revealed diastolic cardiac arrest and marked venous congestion.

The foregoing procedure provides a convenient, standardized basis for the comparative evaluation of the hypotensive activity of groups of compounds. However, the dose-response relationship is obviously distorted to an unknown extent by the residue of previously administered doses. Therefore it was considered desirable to determine the effects of single doses of the most active chalcone of this series (compound A) in a larger group of animals. Compound A injected intravenously in a dose of 4 mg./Kg. produced a slight (20 to 30 mm. Hg) rapid but transient (five to twenty minutes duration) drop in mean arterial blood pressure in both intact ($N = 7$)² and vagally denervated ($N = 3$) mongrel dogs anesthetized with pentobarbital sodium. The reduction in arterial pressure obtained with an intravenous dose of 8 mg./Kg. was not appreciably greater (20 to 40 mm. Hg), however the duration of the depressor response was extended from sixty to one hundred and twenty minutes in both intact

($N = 10$) and vagally denervated dogs ($N = 4$).

Hypotensive Activity in Hypertensive Rats.³—Various methods of inducing experimental hypertension were investigated. The procedure employed in this study was selected on the basis of the production of persistent, moderately severe hypertension in a high percentage of animals operated upon, coupled with a high rate of survival.

Hypertension was induced in male Sherman rats, weighing approximately 175 Gm., by applying a figure-8 ligature, made with coarse thread, to one kidney stripped of fascia; removal of the contralateral kidney two weeks later, and subcutaneous implantation of a single 75-mg. pellet of desoxycorticosterone acetate in the shoulder area. All animals were maintained in constant temperature quarters with free access to Rockland Rat Diet and tap water.

Blood pressure was determined in the unanesthetized rat under standard conditions (7) by the photoelectric tensometer method (8), incorporating minor modifications which have increased accuracy and ease of performance. Only rats developing sustained systolic blood pressure of 170 mm. Hg, or greater, were employed. The animals were randomly separated into experimental groups with comparable pretreatment blood pressures.

The hypotensive activity of compound A in doses of 4 and 8 mg./Kg. injected intraperitoneally, and 8 and 16 mg./Kg. administered orally, was compared in each case with hydralazine (Apresoline) administered in doses $1/10$ as great (i.e., 0.4 and 0.8 mg./Kg., intraperitoneally; 0.8 and 1.6 mg./Kg., per os). All solutions were prepared in distilled water.

Under the conditions of this investigation the blood pressures of chronic hypertensive rats receiving only distilled water orally or intraperitoneally remained very stable when measured 1, 2, 4, and 6 hours after administration. Mild reduction in systolic blood pressure, which was largely dissipated after four hours, followed intraperitoneal injection of 4 mg./Kg. of compound A (Table III). Increase in dosage to 8 mg./Kg. resulted in only slight increase in the degree and duration of depressor activity. Comparable effects were elicited by 16 mg./Kg. of compound A administered orally, indicating a moderate degree of gastrointestinal absorption. It is interesting to note that the intraperitoneal and oral LD₅₀ values of compound A obtained in mice differ by a factor (approximately 4) similar to the ratio between doses of compound A producing equivalent decreases in blood pressure by oral and intraperitoneal routes of administration.

A greater degree and duration of blood pressure reduction in the hypertensive rat was obtained with hydralazine administered orally and intraperitoneally in doses only $1/10$ as large as those of compound A.

Acute Toxicity.—Female Swiss Webster mice (HTF strain) weighing from 18 to 22 Gm. and fasted for twelve hours with free access to water were used throughout these studies. The LD₅₀ values for compound A were determined by the oral, intraperitoneal and intravenous routes of administration. Those animals which succumbed

³ The authors wish to acknowledge the assistance of Mr. Sidney Goldstein during this phase of the investigation.

² N denotes number of animals used.

TABLE II—EFFECTS OF A SERIES OF CHALCONES ON BLOOD PRESSURE, RESPONSE TO EPINEPHRINE, AND CAROTID ARTERY OCCLUSION^a

Compd	N ^c	Dose in mg/Kg ^b																
		1			2			8			16			32				
		BP ^d	Ep ^e	CSR ^f	BP	Ep ^e	CSR ^f	BP	Ep ^e	CSR ^f	BP	Ep ^e	CSR ^f	BP	Ep ^e	CSR ^f	BP	
A	5	0	-32	-28	td ^h	-10	-41	32	-50	-58	61 ^g	-60	-75	2/5	72 ^s	-67	-90	3/5
B	2	0	0	0	0	0	0	td	0	0	td	0	0	1/2	td	0	0	1/2
C	3	0	-8	-15	td	-10	-25	19	-30	-56	11	-48	-90	2/3	50	-67	-90	1/3
D	2	0	0	0	td	0	0	td	0	-10	33	0	-17	1/2	21	-10	-90	1/2
E	2	0	0	0	td	+13	0	td	+21	0	td	+27	-7	1/2	20	0	-90	1/2
F	2	0	0	0	0	0	-15	td	0	-25	td	0	-25	1/2	td	0	-20	1/2
G	2	0	-9	0	td	0	0	td	0	+7	td	+25	0	1/2	td	+25	-37	1/2
H	2	0	0	0	0	0	0	td	0	0	td	0	-22	0	td	-15	-46	2/2
I	3	0	0	-7	0	0	-10	td	0	-22	td	0	-20	2/3	td	+10	-20	1/3
J	2	0	0	0	0	0	+10	td	0	+15	td	-15	+65	0	td	-37	-12	2/2
K	2	0	0	0	0	0	-12	td	0	-17	td	0	-35	0	td	-11	-80	2/2
L	2	0	0	0	0	+11	0	0	+23	0	td	+22	-25	1/2	td	+10	0	1/2
M	3	0	0	-28	td	0	-10	td	0	-67	61	0	-90	2/3	90	0	-90	1/3
N	2	0	0	0	0	0	0	td	0	0	td	0	0	0	td	0	-40	2/2

^a All experiments performed in pentobarbitol sodium anesthetized normotensive vagally denervated mongrel dogs
^b All doses injected intravenously (femoral vein). Succeeding doses injected after BP returned to normal or after interval of twenty minutes if preceding dose had no significant effect
^c N = Number of animals used
^d BP = Mean decrease in blood pressure in mm. Hg (recorded from the femoral artery)
^e Ep = Mean per cent change in response to intravenous epinephrine hydrochloride (0.25 to 0.5 ml of 1:20,000 solution)
^f CSR = Mean per cent change in response to occlusion of the carotid arteries for thirty seconds
^g 1 = 1 fatal (No died No used) ^h td = Transient drop in blood pressure ^s s = Sustained for 60 minutes or more

TABLE III COMPARATIVE HYPOTENSIVE EFFECT OF COMPOUND A AND HYDRALAZINE IN THE HYPERTENSIVE RAT^a

Mean Pretreatment Blood Pressure (Systolic) mm Hg	Drug	Dose mg/kg	Route of Administration	Mean Reduction in Blood Pressure, mm Hg				S.D. mm Hg
				Time in Hours after Administration of Drug				
				1	2	4	6	
230	Compound A	4	i.p.	24	25	11	0	±5
213	Hydralazine	0.4	i.p.	26	27	14	1	
223	Compound A	8	i.p.	28	37	21	3	±3
222	Hydralazine	0.8	i.p.	70	77	50	3	
215	Compound A	8	per os	10	16	7	1	±7
230	Hydralazine	0.8	per os	42	47	22	4	
206	Compound A	16	per os	24	21	7	1	±8
208	Hydralazine	1.6	per os	58	58	25	8	

^a N = 10 in all groups

did so within four hours after intrasophageal or intraperitoneal injection, or within thirty minutes after intravenous injection. In each case, death was preceded by salivation, ataxia, tremor, and clonic convulsions. Immediate postmortem examination revealed marked venous congestion and diastolic cardiac arrest. LD₅₀ values and their limits of error were calculated according to a modification of the method of Epstein and Churchman (9).

The oral LD₅₀ of compound A was found to be 603 mg/Kg (95% Fiducial limits of 537 and 676 mg/Kg), the intraperitoneal LD₅₀ was determined to be 158 mg/Kg (95% Fiducial limits of 148 and 170 mg/Kg); and the intravenous LD₅₀ was calculated to be 40.8 mg/Kg (95% Fiducial limits of 37.2 and 44.7 mg/Kg) in mice.

Administration of equivalent doses of compound A to female Sherman rats (Scott Farms) weighing from 160 to 175 Gm indicated the LD₅₀ values to be within the ranges noted for mice. The intravenous LD₅₀ of compound A obtained in mice closely corresponds to the intravenous doses which produced death in anesthetized dogs during the determination of the hypotensive activity of this molecule.

Reduction of Epinephrine Toxicity in Mice.—The degree and duration of protection provided by compound A against the lethal effect of epinephrine in mice was determined according to the method of Loew and Micetich (10) with minor modification. The doses of compound A employed were 39.5 and 79 mg/Kg (representing 1/4 and 1/2 of the intraperitoneal LD₅₀, respectively), injected intraperitoneally into various groups each composed of 20 mice. These doses of compound A were compared to 10 mg/Kg of tolazoline (Priscoline) hydrochloride injected intraperitoneally. At varying time intervals (15, 30, 60, and 120 minutes) after administration of either saline, compound A or tolazoline, the mice were injected intraperitoneally with a standard dose of 14.4 mg/Kg of epinephrine hydrochloride. This concentration of epinephrine sufficed to kill an average of 85% of the unprotected (saline injected) control animals as indicated by the dotted line in Fig. 1.

Administration of both 1/4 and 1/2 of the intraperitoneal LD₅₀ of compound A reduced the lethal effects of epinephrine at the 15, 30, and 60-minute test periods. This activity was dissipated in two hours and the mortality returned to the control

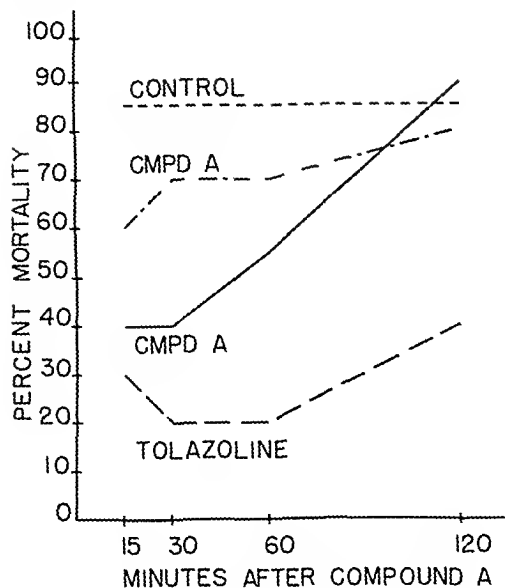


Fig 1—Reduction of epinephrine toxicity in mice. Saline control ---, Compound A 39.5 mg/Kg, 1 p, —, compound A 79 mg/Kg, 1 p, —, Tolazoline 10 mg/Kg, 1 p, —. Each group of 20 mice received epinephrine HCl, 14.4 mg/Kg, 1 p, at the time interval indicated after administration of the test solution

level. A greater degree and duration of protection against the toxic effects of epinephrine was provided by a significantly smaller dose (10 mg/Kg) of tolazoline.

The reduction of epinephrine toxicity in mice with a given compound does not necessarily indicate adrenergic blocking activity (10). Other experiments have demonstrated that compound A possesses marked cardioinhibitory and vasodilator activity, both effects which might account for its ability to decrease the lethal action of epinephrine.

Prolongation of Sleeping Time in Mice.—During the evaluation of the hypotensive activity of compound A in pentobarbital sodium anesthetized dogs, it was consistently noted that the animals remained unconscious for protracted periods without supplementation of the original dose of anesthetic. On this basis it was considered desirable to investigate the effect of this chalcone on the duration of barbiturate induced sleeping time in mice.

Compound A was injected intraperitoneally into varying groups of mice⁴ in doses of 39.5 and 79 mg/Kg (representing $\frac{1}{4}$ and $\frac{1}{2}$ of the intraperitoneal LD_{50} , respectively) ten minutes prior to intraperitoneal administration of either saline (control), 45 mg/Kg of pentobarbital sodium or 112 mg/Kg of hexobarbital sodium. The interval between administration of the barbiturate and re-appearance of the "righting reflex" (animal turns spontaneously over into normal position but may continue to sleep) was recorded as the "sleeping time" (11).

Pretreatment with doses of compound A (39.5 and 79 mg/Kg) which produced only mild ataxia,

markedly prolonged the duration of hypnosis in mice induced by either pentobarbital sodium or hexobarbital sodium (Table IV). "Sleeping time" following administration of 45 mg/Kg of pentobarbital sodium was increased by a factor of 5.8 by prior injection of 79 mg/Kg of compound A without any mortality in the group. The hypnotic effect of hexobarbital sodium, in the dose employed, was also prolonged but to a lesser extent.

This experiment was repeated using the same materials as outlined above, but the procedure was modified by administration of compound A ten minutes after injection of the barbiturates. The prolongation of "sleeping time" following post-treatment with compound A was considerably less than that obtained by pretreatment with comparable doses of the chalcone (results not reported). This difference may be largely attributed to the relatively short duration of action of the hydroxychalcones.

At this time we cannot account for the mechanism by which compound A prolongs the barbiturate hypnosis. Preliminary investigation in this direction indicates a reduction in the rate of barbiturate excretion.

Antihistaminic Activity.—The techniques employed and the results obtained in the evaluation of the antihistaminic and antispasmodic activity of this new series of chalcone derivatives have been previously described in detail by Rossi and Avellino (12), therefore this phase of the investigation will be only briefly discussed in the present report.

The ability of each chalcone in the series to inhibit histamine, acetylcholine, and barium chloride induced spasms was compared with diphenhydramine (Benadryl), atropine, and papaverine on isolated guinea pig ileum according to a modification of the Magnus method. On the basis of the techniques employed, compounds A, C, and D exhibited the greatest antispasmodic activity *in vitro*. The results obtained with these three chalcones on isolated gut are summarized in Table V.

The degree of inhibition of the characteristic depressor response to intravenous histamine in anesthetized cats obtained with various doses of the three most effective chalcones is compared with diphenhydramine in Table VI. These data indicate compounds A, C, and D to be significantly less active than diphenhydramine *in vivo*. It is generally recognized, however, that this procedure frequently does not provide an adequate measure of the potency differences between antihistaminic compounds.

From the data presented in Tables V and VI, it is evident that both the antihistaminic potency and specificity of diphenhydramine are considerably greater than any of the compounds investigated. It may therefore be concluded that this group of chalcones is devoid of significant antihistaminic activity.

DISCUSSION

A new series of chalcone derivatives synthesized as potential hypotensive agents have been evaluated for blood pressure reducing activity in the anesthetized normotensive dog. On the basis of this preliminary investigation, the most effective member of this series, 2-(2-dimethylaminoethoxy) chalcone

⁴ Described in the section on acute toxicity.

TABLE IV.—EFFECT OF COMPOUND A ON PENTOBARBITAL SODIUM AND HEXOBARBITAL SODIUM SLEEPING TIME IN MICE

First Injection	mg /Kg I P	Second Injection 10 Minutes after First Injection	mg /Kg I P	Mean Sleeping Time, Min	Ratio	N ^a
Compound A	39.5 ^b	Saline	.	0	...	40
Compound A	79.0 ^c	Saline	.	0	...	40
Saline		Pentobarbital Sodium	45	17	1.0	40
Compound A	39.5	Pentobarbital Sodium	45	37	2.2	20
Compound A	79.0	Pentobarbital Sodium	45	98	5.8	20
Saline		Hexobarbital Sodium	112	29	1.0	40
Compound A	39.5	Hexobarbital Sodium	112	51	1.7	20
Compound A	79.0	Hexobarbital Sodium	112	70	2.4	20

^a N = number of animals used
^b 39.5 mg /Kg is equivalent to 1/4 of the i.p. LD₅₀ of compound A in mice
^c 79.0 mg /Kg is equivalent to 1/2 of the i.p. LD₅₀ of compound A in mice

TABLE V —AVERAGE PER CENT INHIBITION OF THE CONTRACTILE RESPONSE TO HISTAMINE, ACETYLCHOLINE, AND BARIUM CHLORIDE IN THE ISOLATED GUINEA PIG ILEUM BY GRADED DOSES OF THE MOST ACTIVE CHALCONES

Histamine, 10 mg /100 ml				
Compound	Concn. 0.0001	of Antagonist 0.001	in mg /100 ml 0.01 0.1	
A	0	0	0	10
C	0	0	8	18
D	0	0	0	14
Diphenhydramine	0	73	85	
Acetylcholine, 100 mg /100 ml				
Compound	Concn. 0.01	of Antagonist 0.1	in mg /100 ml 1.0	
A	0	0	0	51
C	0	58	97	
D	0	60	96	
Diphenhydramine	0	70	100	
Atropine	100			
Barium Chloride, 500 mg./100 ml				
Compound	Concn. 0.5	of Antagonist 5.0	in mg /100 ml 50.0	
A	66	97	100	
C	50	100	100	
D	66	98	100	
Diphenhydramine	56	100	100	
Papaverine	10	95	100	

^a Each figure represents the average of determinations made on three isolated muscle strips.

citrate (designated as compound A) was subjected to further study of depressor activity in the dog and in the unanesthetized hypertensive rat. Compound A, administered orally and parenterally, provided moderate reduction of blood pressure but for a period of time insufficient to suggest possible clinical usefulness. The mechanism of compound A in reducing blood pressure has not been fully elucidated, but appears to be the result of direct smooth muscle relaxation in addition to incomplete adrenergic blockade.

The ability of the fourteen new chalcones to inhibit histamine, acetylcholine, and barium chloride

TABLE VI —AVERAGE PER CENT INHIBITION OF THE DEPRESSOR RESPONSE TO HISTAMINE IN THE ANESTHETIZED CAT BY GRADED DOSES OF THE MOST ACTIVE CHALCONES

Histamine, 1 mg./Kg. Intravenously				
Compound	Dose of Antagonist in mg /Kg. Intravenously			
	1	2	3	4
A	0 ^a	0	0	10
C	0	11	23	38
D	0	0	0	0
Diphenhydramine				68

^a Each figure represents the average of determinations made on two or more animals.

induced spasm was compared with diphenhydramine, atropine, and papaverine on isolated guinea pig ileum. The three chalcones (compounds A, C, and D) found most effective *in vitro* were also examined for their ability to reduce the histamine depressor response in the anesthetized cat. Although antihistaminic and antispasmodic activity was manifested by certain members of this series, the potency and duration of activity was significantly less than that displayed by presently available compounds in these categories.

The series of hydroxychalcones which are the subject of this report represented an interesting theoretical approach to the synthesis of hypotensive agents. The derivatives examined shared the major disadvantages of low potency, lack of specificity, and short duration of action. Synthesis of additional members of this series may provide chalcones with greater hypotensive or antihistaminic effectiveness.

SUMMARY

1. A series of fourteen new chalcone derivatives were evaluated with regard to their hypotensive potency and ability to inhibit the pressor responses to epinephrine and occlusion of the carotid arteries in anesthetized normotensive dogs,

2. The blood pressure reducing activity of the most effective member of the series, 2-(2-dimethylaminoethoxy) chalcone citrate (compound A), was compared with hydralazine in the unanesthetized hypertensive rat.

3. The acute toxicity of compound A and its influence on epinephrine toxicity and barbiturate induced "sleeping time" were determined in mice.

4. On the basis of structural similarity to certain antihistamines, the activity of this series of chalcones against histamine, acetylcholine, and barium chloride induced spasms was compared with diphenhydramine, atropine, and papaverine on isolated guinea pig ileum. Three of the most effective chalcones (compounds A, C, and D) were examined for their ability to inhibit the histamine depressor response in anesthetized cats.

5. The chalcones investigated in this study

exhibited relatively low specificity, low potency, and transient duration of action. Synthesis of additional chalcones, with consideration given to the activities reported, is recommended.

REFERENCES

- (1) Clark, W. G., *Chem. Eng. News*, 31, 1401(1953).
- (2) Beiler, J. M., *Arch. Biochem.*, 26, 72(1950).
- (3) Beiler, J. M., and Martin, G. J., *J. Biol. Chem.*, 192, 831(1951).
- (4) Beiler, J. M., Graff, M., and Martin, G. J., *Am. J. Digest. Diseases*, 19, 333(1952).
- (5) Bartlett, G. R., *J. Pharmacol. Exptl. Therap.*, 93, 329(1948).
- (6) Packman, A. M., and Rubin, N., Report based on Doctorate Thesis, Philadelphia College of Pharmacy and Science, 1956, in press.
- (7) Sturtevant, F. M., *Proc. Soc. Exptl. Biol. Med.*, 84, 101(1953).
- (8) Kersten, H., Grosene, W. G., Jr., Ablondi, F., and Subba Row, Y., *J. Lab. Clin. Med.*, 32, 1090(1947).
- (9) Epstein, B., and Churchman, C. W., *Ann. Math. Stat.*, 15, 90(1944).
- (10) Loew, E. R., and Micetich, A., *J. Pharmacol. Exptl. Therap.*, 93, 434(1948).
- (11) Ambrus, J. L., Ambrus, C. M., Leonard, C. A., Moser, C. E., and Harrison, J. W. E., *THIS JOURNAL*, 41, 606(1952).
- (12) Rossi, G. V., and Avellino, J. D., *Am. J. Pharm.*, 129, 321(1957).

Anticonvulsant Drug Combinations: Diphenylhydantoin Combined with Other Antiepileptics*

By L. C. WEAVER,† E. A. SWINYARD, and L. S. GOODMAN

Methods are presented for the study of drug combinations in which one component is active by the anticonvulsant test and both components are active by the neurotoxicity test. The effects of Dilantin on anti-Metrazol activity and neurotoxicity of five clinically useful antiepileptics were determined in mice and protective indices calculated. The anti-Metrazol activity of two combinations of Dilantin-Phenurone was significantly greater than that of Phenurone alone. The minimal neurotoxic doses of all combinations of Dilantin-Tridione and Dilantin-Mesantoin were significantly larger than those calculated on the basis of similar joint action of toxicities. The margin of safety of one combination of Dilantin-Mesantoin was significantly increased over that of the active component alone.

A PREVIOUS PUBLICATION (1) described a method for the laboratory evaluation of combinations of two antiepileptic agents, both of which are active by the assay procedure employed; the anticonvulsant activity of various combinations of diphenylhydantoin (Dilantin) and phenobarbital were determined by the maximal electroshock seizure test. These studies have now been extended to anticonvulsant drug combinations in which only one component of the

mixture is active by the test employed. Dilantin, a compound ineffective against pentylenetetrazol (Metrazol)-induced seizures (2, 3), was combined with agents known to be effective against this convulsant, and the anti-Metrazol activity of the mixtures was then determined. In addition, the minimal neurotoxic dose for each combination was measured. The results obtained provide the basis for this report.

METHODS

Assay Procedures.—Phenobarbital sodium, phenacemide (Phenurone¹), trimethadione (Tridione¹), methylphenylethylhydantoin (Mesan-

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¹ Supplied by Abbott Laboratories.

toin²), and mephobarbital (Mebaral³), alone and in combination with diphenylhydantoin (Dilantin⁴) sodium, were tested in mice for ability to prevent pentylenetetrazol (Metrazol⁵)-induced seizures and for neurotoxicity. The quantity of each active drug which protected 50% of mice from Metrazol (ED_{50}) was combined with 3, 6, and 12 mg./Kg. of the inactive drug (Dilantin); in two experiments Phenurone and Tridione were combined with 21 and 30 mg./Kg. of Dilantin, respectively. Each combination was evaluated as a new drug. All drugs and all drug combinations were administered orally in 10% acacia solution. Male albino mice (Carworth Farms, CF #1 strain), 17 to 37 Gm. wt. were used as experimental animals; they were allowed free access to food (Purina Laboratory Chow) and water except during the actual test period. Anticonvulsant activity was determined by the Metrazol seizure threshold (Met.) test (85 mg./Kg. administered subcutaneously as a 0.5% aqueous solution) and neurotoxicity was determined on the basis of signs of minimal overt neurological deficit. The details of the Met. test and the end points employed for the determination of acute neurotoxicity in mice have been described elsewhere (4). All tests were performed at the time of peak activity of the active component (see footnotes, Table I).

In the determination of anticonvulsant activity and neurotoxicity, groups of 8 to 12 mice were tested with various doses of drug or drug combination until at least three points were established between the limits of complete protection or toxicity and no protection or toxicity. The results obtained were plotted on logarithmic probability paper and regression lines fitted to the plotted points by the method of maximum likelihood. The dose of drug or drug combination producing the desired end point (protection or neurotoxicity) in 50% of animals (ED_{50} or TD_{50}) was computed. The regression lines for anti-Metrazol activity and neurotoxicity of each drug and drug combination were tested for parallelism for the Chi-square method described by Finney (5), and protective indices (TD_{50}/ED_{50}) were calculated. In those instances where fiducial limits were desired, a method described by Finney (5) was utilized.

Statistical Analysis.—The values obtained by the Met. test were analyzed for potentiation or antagonism of anticonvulsant activity. A significant change in anti-Metrazol activity was considered to have occurred when the 95% fiducial limits of the active component in the combination were outside those of the active component alone. When significantly less of the active drug was required for 50% protection, the result was considered to represent potentiation; conversely, when significantly more of the active drug was required, the result was considered to represent antagonism. Since all drugs and combinations used in these studies exhibited toxicity in appropriate doses, the neurotoxicity values obtained were statistically analyzed by the method described by Finney (5) for any departure from the prediction of similar joint action.

RESULTS

The data obtained are summarized in Table I. The ED_{50} 's calculated by the probit method of analysis are listed under the column marked anti-Metrazol activity. Under the general title neurotoxicity are listed the calculated TD_{50} 's, observed TD_{50} 's, toxicity ratios, mean probit differences, and P values for the mean probit differences. The calculated TD_{50} 's were mathematically derived by the probit method of Finney (5) for similar joint action, whereas the observed TD_{50} 's were determined experimentally. The toxicity ratios were derived by dividing the calculated toxicity value by the observed toxicity value; numbers below 1.00 indicate an effect less than that calculated; numbers above 1.00 indicate an effect greater than that calculated. The mean probit difference is the constant vertical difference between the two parallel probit regression lines; it measures only the difference in effect of equal doses; it does not compare the magnitudes of equally effective doses. Comparison of the mean probit difference with its standard error provides a test for the significance of the departure from the prediction of simple addition on the basis of similar joint action; positive values of the mean probit difference indicate that the observed toxicity is greater than predicted; negative values, less than predicted. The P value denotes the significance of the departure. The protective index (observed TD_{50} divided by the ED_{50}) is shown in the last column. Because the toxicity regression lines for Phenurone and Phenurone-Dilantin combinations were not parallel, it was not possible to analyze the data by the method of probit analysis; therefore, these data were analyzed by the same method as used for anticonvulsant activity.

A study of Table I reveals that, except for two combinations with Phenurone, Dilantin had no significant effect on the anti-Metrazol activity of the five drugs tested. In the two exceptions, the amount of Phenurone required to protect 50% of animals was significantly reduced by the addition of small amounts of Dilantin. For example, the ED_{50} and 95% fiducial limits of the combination composed of 16.7% Dilantin and 83.3% Phenurone were 74.1 (56.0–98.2) mg./Kg., whereas for Phenurone alone they were 119.7 (111.2–129.5) mg./Kg. Thus, the anti-Metrazol activity of this particular combination was increased by approximately 38% over that of Phenurone alone.

The neurotoxicity of Mebaral, Mesantoin, and Tridione was decreased below that expected (based on the prediction of similar joint action) by the addition of small amounts of Dilantin, whereas the neurotoxicity of phenobarbital was not altered by such addition. Although no quantitative determination could be made of the effect of Dilantin on the neurotoxicity of Phenurone, the fact that the margins of safety (protective indices) were not significantly changed suggests that the increase in neurotoxicity merely paralleled the increase in anti-Metrazol activity.

Except for one combination (Dilantin 6.5%–Mesantoin 93.5%), there was no significant alteration in the protective indices of the seventeen combinations tested. The protective index and 95% fiducial limits for this particular Dilantin-

² Supplied by Sandoz Chemical Works, Inc.

³ Supplied by Sterling-Winthrop Research Institute.

⁴ Supplied by Parke-Davis Company.

⁵ Supplied by Billhuber-Knoll Corporation.

TABLE I.—EFFECT OF DILANTIN SODIUM ON THE ANTI-METRAZOL ACTIVITY, NEUROTOXICITY, AND PROTECTIVE INDEX OF FIVE ANTIEPILEPTIC DRUGS

Drug or Drug Combination		Anti-Metrazol Activity, ED ₅₀ , Active Component, mg/Kg	Neurotoxicity				P for Mean Probit Difference	Protective Index
Active Component	% ^a		Calculated TD ₅₀ , mg/Kg	Observed TD ₅₀ , mg/Kg	Toxicity Ratio	Mean Probit Difference ± S E		
Phenobarbital Sodium	100 0	20 3 (18 9-21 8)	.	78 6	.	.	.	3 87 (3 40-4 42)
	87 2	15 9 (13 1-19 3)	80 8	75 7	1 07	+0 42 ± 0 41	0 1	4 76
	77 4	19 0 (15 9-21 8)	82 6	84 1	0 98	-0 12 ± 0 41	0 7	4 43
	63 0	17 6 (15 7-19 7)	85 0	84 4	1 81	+0 06 ± 0 40	0 7	4 80 (3 90-5 90)
Mebamal	100 0	19 8 (16 8-23 3)	.	64 1	.	.	.	3 24 (2 57-4 07)
	87.5	18 0 (14 8-22 0)	66 6	83 2	0 80	-0 73 ± 0 39	0 05	4 62 (3 39-6 31)
	77 8	16 0 (13 5-19 0)	68 1	83 2	0 82	-0 66 ± 0 38	0 05	5 19 (4 05-6 65)
	63 6	19 1 (17 3-21 1)	72 2	87 9	0 82	-0 64 ± 0 32	0 05	4.62 (3 25-6 59)
Mesantoin	100 0	42 8 (35 7-51 2)	.	106 0	.	.	.	2 48 (1 96-3 13)
	93 5	34 3 (28 8-40 8)	106 3	143 9	0 74	-1 90 ± 0 48	0 001	4 20 (3 43-5 14)
	87.8	45 9 (41 3-51 1)	106 4	142.7	0 75	-1 83 ± 0 43	0 001	3 11
	78 3	51 6 (41 0-65 0)	106 7	148 5	0 72	-2 06 ± 0 43	0 001	2 89
Tridione	100 0	371 6 (342 8-402 7)	...	774 1	.	.	.	2 08 (1 91-2 33)
	99 2	363 4 (330 4-399 1)	733 0	992 6	0 74	-2 03 ± 0 36	0 001	2 73
	98 5	381 6 (261 3-558 4)	696 5	1070 0	0 65	-2 88 ± 0 32	0 001	2 80 (1 91-4.11)
	96 9	372 3 (314 0-441 5)	634 3	1062 8	0 60	-3 46 ± 0 29	0 001	2 85 (2 37-3 43)
Phenurone	100 0	331 8 (171 8-641 3)	507 0	858 6	0 59	-3 53 ± 0 34	0 001	2 59
	97 6	119 7 (111 2-129 5)	.	372 6 ^c (344 4-402 7)	.	.	.	3 11 (2 61-3 70)
	95 2	100 6 (90 4-112 3)	.	372 1 (342 0-405 5)	.	.	.	3 70
	90 9	102 1 (83 8-124 5)	.	313 3 (270 4-363 1)	.	.	.	3 08
Dilantin Sodium	83 3	94 2 (83 8-105 9)	.	352 2 (311 9-398 1)	.	.	.	3 74
	56 0	74 1 (56 0-98 2)	.	265 2 (226 7-311 2)	.	.	.	3 59

^a Made up to 100% with Dilantin sodium. ^b See text for discussion.^c 95% confidence limits. ^d Inactive by this test.^e Control TD₅₀ for Dilantin Sodium were determined at the following times which represent the times of peak effect for the orally administered active compound given in parentheses: 3 0 hr (phenobarbital sodium) 99 6 mg/Kg, 2 5 hr (Mebamal) 93 3 mg/Kg, 1 5 hr (Mesantoin) 109 5 mg/Kg, 2 0 hr (Tridione) 94 4 mg/Kg, and 1 0 hr (Phenurone) 120 mg/Kg.

Mesantoin combination were 4 20 (3 43-5 14), whereas the comparable values for Mesantoin alone were 2 48 (1 96-3 13); this represents approximately a 70% improvement in margin of safety over that of Mesantoin alone.

DISCUSSION

The difficulties encountered in the quantitative analysis of the effects of drug combinations composed of two or more components, both of which are active by the test employed, have been discussed in previous publications from our laboratories (1, 6).

Fortunately, when only one component of the mixture is active by the assay procedure employed, the quantitative analysis of the combined drug effects is less complex. Indeed, such combinations can be readily assayed and the results directly compared with similar data obtained with the active compound alone. Thus, in the present studies, a significant alteration in the anti-Metrazol activity of combinations of the active drug and Dilantin (a drug inactive against Metrazol) was considered to indicate potentiation or antagonism of drug action, depending on the direction of the alteration.

It is noteworthy that the anti-Metrazol activity of Phenurone increased as the dose of Dilantin increased. This suggests that the potentiating effects of Dilantin on the anti-Metrazol activity of Phenurone was dose-dependent, and indicates that even higher doses of Dilantin should be investigated. However, since there was no significant improvement in the protective index after a four-fold increase in the dose of Dilantin and because of the stimulant effects known to occur with high doses of Dilantin, it was not considered advisable to extend the Dilantin dose range.

When compared on the basis of neurotoxicities, it may be seen that the results obtained with combinations of Dilantin-phenobarbital and Dilantin-Mebaral agree with the prediction of similar joint action. According to the previously described interpretations (1), the combinations composed of Mesantoin and Tridione in combination with Dilantin exhibit antagonism of toxicity. Although the Dilantin-Phenurone toxicity data could not be evaluated by the probit method because the regression lines were not parallel, the data indicate a trend toward increased toxicity which paralleled the increase in anti-Metrazol activity.

As previously suggested by Loewe (6), it is more important to study the ratio between the intensities of various effects of the same combination, i. e., to know whether these ratios (margins of safety or protective indices) assume a large or smaller value for the combinations than for the components. The combination composed of Dilantin 6.5%-Mesantoin 93.5% exhibited a protective index which was approximately 70% higher than that for Mesantoin alone.

Since a major objective of this study was to present the experimental design and the statistical analysis for evaluating drug combinations in which only one component of the mixture is active by the test employed, no concerted effort was made to cover the entire spectrum of activity of each component or combination. Hence, speculation on the laboratory and clinical implications of these data is not justified. Nevertheless, the results obtained with combinations of Dilantin-Phenurone and Dilantin-Mesantoin are sufficiently promising to warrant their further study.

SUMMARY

Methods are presented for the study of anti-convulsant drug combinations in which only one

component is active by the assay test employed and both components are active by the neurotoxicity test. To illustrate this procedure, small amounts of Dilantin, an agent ineffective against Metrazol-induced seizures, were combined with known effective doses of phenobarbital sodium, Mebaral, Mesantoin, Tridione, and Phenurone, and the anti-Metrazol activity and neurotoxicity of each combination were determined; in addition, the protective indices were calculated. The anticonvulsant data obtained were analyzed for evidence of antagonism or potentiation of anti-Metrazol activity, whereas the toxicity data were analyzed for any departure from the prediction of similar joint action.

The results obtained may be summarized as follows:

1. Dilantin significantly increased the anti-Metrazol activity of the two combinations which contained 90.9 and 83.3% Phenurone, by approximately 27 and 38%, respectively; but Dilantin had no effect on the activity of phenobarbital, Mebaral, Tridione, or Mesantoin.

2. The neurotoxicities were significantly decreased in all combinations of Dilantin with Tridione, Mesantoin, and Mebaral, unchanged in all combinations of Dilantin with phenobarbital, and apparently increased in all combinations of Dilantin with Phenurone although nonparallelism of regression lines precluded the quantitative evaluation of this latter set of combinations.

3. One of the seventeen combinations tested, Dilantin 6.5% with Mesantoin 93.5%, exhibited a 70% increase in margin of safety.

REFERENCES

- (1) Weaver, L. C., Swinyard, E. A., Woodbury, L. A., and Goodman, L. S., *J. Pharmacol. Exptl. Therap.*, **113**, 359 (1955).
- (2) Goodman, L., and Lih, B., *ibid.*, **72**, 18 (1941).
- (3) Chen, G., and Ensor, C. R., *J. Lab. Clin. Med.*, **41**, 78 (1953).
- (4) Swinyard, E. A., Brown, W. C., and Goodman, L. S., *J. Pharmacol. Exptl. Therap.*, **106**, 319 (1952).
- (5) Finney, D. J., "Probit Analysis," 2nd ed., Cambridge University Press, London, 1952.
- (6) Loewe, S., *Arzneimittel-Forsch.*, **3**, 285 (1953).

Study of Stability of Sulfadiazine Sodium Injection IV.*

The Determination of Oxygen Uptake of Sulfadiazine Sodium with Antioxidants in Solution

By CHARLES J. SWARTZ† and JOHN AUTIAN‡

The addition of certain antioxidants to ampuls of sulfadiazine sodium solution prepared in a nitrogen atmosphere retards the formation of color. The efficiency of various antioxidants to inhibit the development of color was investigated by determining the ability of these agents to utilize available oxygen in sulfadiazine sodium solutions at various hydrogen ion concentrations. Quantitative estimation of oxygen uptake was achieved by the utilization of a Warburg Constant Volume Respirometer. Ascorbic acid, *p*-aminophenol, hydroquinone, sodium formaldehyde sulfoxylate, sodium sulfite, and *tert*-butyl hydroquinone were the antioxidants evaluated. Oxygen consumption data were correlated with actual formation of color in ampuls autoclaved for prolonged periods.

IT WAS OBSERVED in earlier studies (1, 2) that the addition of antioxidants to ampuls of sulfadiazine sodium, sealed under an atmosphere of nitrogen, prevented or delayed the formation of color in these solutions. The antioxidants varied in their ability to hinder this coloration. The solvent, polyethylene glycol, also seemed to have some activity in retarding color formation (2).

It was, therefore, decided to measure the amount of oxygen consumed by solutions of sulfadiazine sodium with antioxidants employing a Warburg apparatus. These values were then correlated with the ability of the various agents to prevent color formation in ampuls subjected to prolonged periods of autoclaving.

EXPERIMENTAL

Determination of Oxygen Consumption.—Oxygen consumption was determined with the aid of a Warburg apparatus utilizing standard procedures as described by Umbreit, *et al.* (3). Single side-arm Warburg flasks of approximately 15-ml. volume were employed in all tests. Fluid volume was kept at 4.0 ml. throughout.

The experimental flasks contained 3.0 ml. of 25.0% sulfadiazine sodium solution buffered at pH 9.75 and 1.0 ml. of a 1.0% solution of the antioxidant under study. Control flasks were prepared containing 3.0 ml. of distilled water and 1.0 ml. of antioxidant. Flasks containing 3.0 ml. of a buffer solu-

tion of pH 9.75 and 1.0 ml. of antioxidant were also prepared.

Oxygen consumption was determined at a temperature of $50.0 \pm 0.02^\circ$ in an atmosphere of air. Preliminary tests indicated that higher oxygen tensions were not suitable for these studies since the reaction proceeded too rapidly. After a fifteen minute equilibration period, readings were recorded at ten minute intervals for three hours. From the readings obtained, the corresponding volume of oxygen consumed by the contents of each flask was calculated in the usual fashion.

Antioxidants which were evaluated in these experiments included: sodium sulfite, sodium formaldehyde sulfoxylate, ascorbic acid, hydroquinone, *tert*-butyl hydroquinone, and *p*-aminophenol. The following combinations of agents were also tested: sodium sulfite and ascorbic acid, sodium formaldehyde sulfoxylate and ascorbic acid, and citric acid with each of the above mentioned three.

Preparation of Ampuls for Autoclaving.—Twenty-milliliter clear glass ampuls Type 2 were used throughout the study. Aqueous solutions of sulfadiazine sodium, buffered at pH 9.75, were prepared containing the antioxidants (in 0.05% concentration) listed in Table 1. The use of hydroquinone, *t*-butyl hydroquinone and *p*-aminophenol was not feasible in this phase of the study as the oxidized forms of the above three agents are highly colored. Consequently, these antioxidants could not be adequately evaluated.

Fifteen milliliters of solution was placed in each ampul and triplicate determinations were made on each series. The ampuls were placed in an electric steam pressure sterilizer (pressure cooker type) after being sealed in the presence of air. Per cent transmittance readings were recorded for these freshly prepared samples utilizing a Beckman Model C colorimeter with a prefocused tungsten lamp and a Corning 4010 green filter. The pressure was regulated to 15–16 p.s.i. A period of twelve hours was found to be adequate to allow the reactions to occur after which the ampuls were removed from the sterilizer. Per cent transmittance of the autoclaved solutions was measured after the contents of the ampuls had returned to room temperature.

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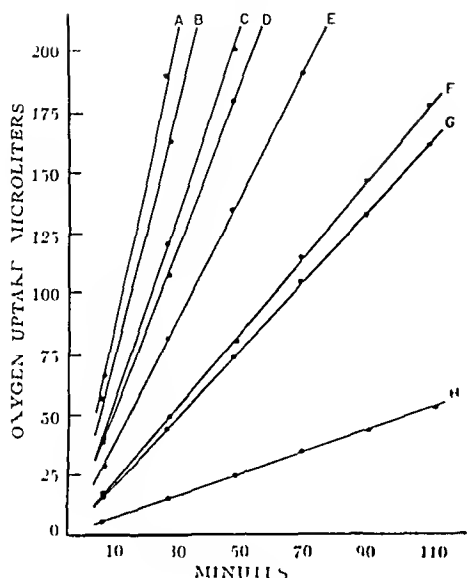


Fig 1—Oxygen uptake data for solutions of sulfadiazine sodium 18.75% with various antioxidants. Concentration of each antioxidant 0.25%. A—Sodium Sulfite, B—Hydroquinone, C—*p*-Amino phenol, D—Sodium Formaldehyde Sulfoxylate with Ascorbic Acid, E—Ascorbic Acid, F—Sodium Sulfite with Ascorbic Acid, G—*t*-Butyl Hydroquinone, H—Sodium Formaldehyde Sulfoxylate

RESULTS AND DISCUSSION

The oxygen uptake for each solution was plotted against time in minutes and appears in Fig 1. It will be noticed that all the curves are smooth in the range studied and that the slope of the lines may be used to indicate the protective effect that these antioxidants have upon sulfadiazine sodium in aqueous solution. A review of the slopes points to the fact that sodium sulfite (A) had the greatest antioxidant effect in comparison to the other agents used in this evaluation. Hydroquinone (B), *p*-amino phenol (C), sodium formaldehyde sulfoxylate with ascorbic acid (D), ascorbic acid (E), sodium sulfite with ascorbic acid (F), and *tert*-butyl hydroquinone (G) each had a slower rate of oxygen uptake while sodium formaldehyde sulfoxylate (H) had the slowest. Since a number of these antioxidants, themselves, upon oxidation produced coloration, they were removed from further consideration. An antioxidant synergist, citric acid, although reported to enhance the action of numerous antioxidants (4), proved ineffectual in the systems studied. It was felt that this experiment would not serve a very useful purpose unless the "oxygen uptake" could be related to the practical aspects of the problem. With this point in view, Fig 2 was prepared. A quantitative relationship could not be observed, but a satisfactory qualitative comparison was possible. For example, when a system (Ampul A in Fig 2) composed of sulfadiazine sodium and sodium sulfite in an aqueous medium was autoclaved for twelve hours, it produced less coloration than the other systems as measured photometrically. This same system in the Warburg experiments con-

TABLE I—PER CENT TRANSMITTANCE OF SULFADIAZINE SODIUM WITH VARIOUS ANTIOXIDANTS AFTER TWELVE HOURS IN AUTOCLAVE AT 15 PSI

Ampul ^a	Contents		Transmittance %	
			Initial	Final
A	Sulfadiazine sodium	18.75%	100	95
	Sodium sulfite	0.05%		
B	Sulfadiazine sodium	18.57%	100	94
	Sodium formaldehyde sulfoxylate	0.025%		
C	Sulfadiazine sodium	18.75%	100	89
	Ascorbic acid	0.05%		
D	Sulfadiazine sodium	18.75%	100	83
	Sodium sulfite	0.025%		
	Ascorbic acid	0.025%	100	76
E	Sulfadiazine sodium	18.75%		
	Sodium formaldehyde sulfoxylate	0.05%	100	62
F	Sulfadiazine sodium	18.75%		

^a Letters refer to samples in Fig 2

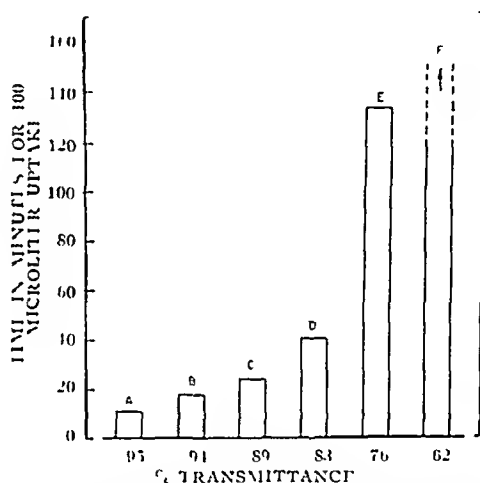


Fig 2—Graph illustrating relation between rate of oxygen uptake vs formation of color in ampuls of sulfadiazine sodium (18.75%) with antioxidants. F = control sulfadiazine sodium

sumed 100 microliters of oxygen in the shortest time period (approximately ten minutes). In Ampul B containing two antioxidants (sodium formaldehyde sulfoxylate and ascorbic acid), a more intense color was produced than in Ampul A and the time for oxygen consumption of 100 microliters was increased. This type of relationship was progressively increased (more color and greater time period for uptake of oxygen) with Ampuls C, D, and E respectively. The control solution (Ampul F), sulfadiazine sodium alone, produced the most coloration. Since the oxygen utilization of sulfadiazine is infinitely slow, it could not be measured by the techniques employed.

Figure 2 also demonstrates the synergistic effect that ascorbic acid had upon sodium formaldehyde sulfoxylate. The per cent transmittance of sodium sulfadiazine in the presence of sodium formaldehyde sulfoxylate was 76% and this rose to 94% when ascorbic acid was included in the system. All reactions containing the buffered sulfadiazine sodium solutions showed increased activity over con-

trol runs. This action cannot be ascribed to ionic strength or pH factors as experiments investigating each of these yielded negative results. Unreported studies also disclosed that if the concentration of antioxidant was increased, no coloration appeared, after twelve hours of autoclaving, in any of the ampuls.

SUMMARY

Oxygen uptake studies utilizing a Warburg Constant Volume Respirometer were conducted on solutions of sulfadiazine sodium with the addition of various antioxidant agents.

The rate of oxygen consumption in these solutions was shown to be directly related to the

ability of the antioxidant to deter color formation in ampuls of sulfadiazine sodium solutions. The tests showed sodium sulfite to have the greatest activity in the system under the conditions of study. A combination of sodium formaldehyde sulfoxylate and ascorbic acid was shown to be almost equally effective.

REFERENCES

- (1) Hom, F. S., and Autian, J., *THIS JOURNAL*, **45**, 608 (1956).
- (2) Hom, F. S., and Autian, J., *Bull. Am. Soc. Hosp. Pharmacists*, **14**, 177 (1957).
- (3) Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques and Tissue Metabolism," Burgess Publishing Co., Minneapolis, 1949.
- (4) Sturton, A. J., Turer, J., and Riemenschneider, R. W., *Oil & Soap*, **22**, 81 (1945).

The Adsorption of Enterobacterial Endotoxins by Activated Attapulgit^{*}

By ERWIN NETER[†] and EUGENE A. GORZYNSKI[‡]

The effect of activated attapulgit upon endotoxins (lipopolysaccharides) of enterobacterial pathogens (enteropathogenic *Escherichia coli* and *Shigella sonnei*) was studied by means of the enterobacterial hemolysis and hemolysis inhibition tests and *in vivo* experiments in mice. It was found that activated attapulgit adsorbs the endotoxins as demonstrated by inhibition of enterobacterial hemolysis, elimination of the specific inhibitory effect of the antigens in hemolysis inhibition tests, and by the abolishment of toxicity in mice. The significance of the findings is discussed with particular reference to their bearing upon the clinical use of activated attapulgit in localized intestinal infections and other syndromes associated with these endotoxins.

RECENT STUDIES by Barr and Arnista (1) have revealed that activated attapulgit adsorbs diphtheria toxin and is approximately five times as effective as kaolin. The question arises as to whether this material also adsorbs endotoxins of enteric bacteria. To this end, the present study was carried out, utilizing the enterobacterial hemolysis and hemolysis inhibition tests and *in vivo* toxicity determinations as indicator systems.

The endotoxins of enteric bacteria, such as *Escherichia (E.) coli*, salmonellae, and shigellae, are composed of polysaccharide, lipid, and protein. These complexes, like the lipopolysaccharide fractions thereof, possess toxicity in mice and other animals, are extraordinarily pyrogenic, profoundly affect natural resistance to unrelated

challenge infections, and act as antigens (haptens) in various serologic procedures. It is the polysaccharide that determines the antigenic characteristics of the complex. Recent studies suggest that toxicity and pyrogenicity are associated with the lipid component (2, 3). Studies from our laboratories have revealed that these enterobacterial lipopolysaccharides readily become attached to red blood cells and thus endow the latter with a newly acquired serologic specificity (4-6). As a result, these modified erythrocytes are specifically agglutinated in the presence of homologous antibodies, and hemolysis occurs upon the addition of antibodies and guinea pig complement, notably when sheep red blood cells are employed. These hemagglutination and hemolysis reactions have proved to be considerably more sensitive for the demonstration of homologous antibodies than the conventional bacterial agglutination procedures. Specific hemolysis is inhibited if antigen is added to the antiserum prior to the addition of complement and erythrocytes modified by the corresponding antigen. The latter procedure, referred to as hemolysis inhibition test, allows the deter-

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mination of lipopolysaccharide in amounts as small as 0.006 µg. (7). In view of the important biologic activities of these endotoxins it was deemed desirable to determine the adsorptive capacity of activated attapulgit upon these lipopolysaccharides. The results of this investigation are embodied in the present report.

MATERIALS AND METHODS

Activated attapulgit was obtained from Wyeth Laboratories and dissolved just prior to use in phosphate hemagglutination buffer (pH 7.2-7.3) (Difco Laboratories).

As antigens, supernates of boiled agar grown cultures of *E. coli* 111:B4 and *Shigella* (*S.*) *sonnei* as well as purified lipopolysaccharides of *E. coli* 111:B4, 0127:B8 and *S. sonnei* were used. The two former lipopolysaccharides were obtained from Difco Laboratories and the latter through the kindness of Professor O. Westphal, Freiburg, Germany. All antigens were dissolved in phosphate buffer.

Mixtures of antigens and activated attapulgit and buffer, respectively, were prepared and incubated in a water bath at 37°, for one hour. The supernates of the mixtures (refrigerated centrifuge) were then used for modification of sheep red blood cells for the hemolysis tests and as antigens in the hemolysis inhibition tests. These methods have been described in detail elsewhere (4, 5, 8).

For the toxicity studies *E. coli* 127:B8 lipopolysaccharide was employed. The endotoxin was mixed with activated attapulgit and buffer, respectively. The mixtures were incubated in a water bath at 37° for one hour and centrifuged. White mice, weighting approximately 20 Gm., were injected intraperitoneally with 1 ml. each of the supernates. The animals were observed for a period of six days and fatal reactions recorded daily.

RESULTS

In the first series of experiments the effect of activated attapulgit on *E. coli* 0111:B4 lipopolysaccharide was determined, using the *E. coli* hemolysis test as indicator system. To this end, 5 µg./ml. of lipopolysaccharide was mixed with activated attapulgit in concentrations ranging from 17 to 5000 µg./ml. and buffer for control purposes. The mixtures were incubated, centrifuged, and supernates tested for remaining lipopolysaccharide by treatment of sheep red cells. The latter were washed and then added to *E. coli* 0111:B4 antiserum in serial dilutions and guinea pig complement. The resulting hemolysis was read and the results are recorded in Table I.

It can be seen from this table that activated attapulgit in concentrations of 500 µg./ml. and higher adsorbed enough lipopolysaccharide to prevent subsequent hemolysis completely and that 170 µg./ml. of this adsorbent reduced the amount of lipopolysaccharide sufficiently to produce partial inhibition of hemolysis. Similar results were obtained in experiments with *S. sonnei* lipopolysaccharide as well as with erden antigens.

The conclusion that activated attapulgit adsorbs endotoxins (lipopolysaccharides) is substantiated further by the results of experiments utilizing hemolysis inhibition as the indicator system. In these experiments various amounts of *E. coli* 0111:B4 lipopolysaccharide (1, 5, 25 µg./ml.) were mixed with activated attapulgit in concentrations of 100 to 10,000 µg./ml. and buffer for control purposes. The mixtures were incubated and centrifuged. The supernatant fluids were then used in hemolysis inhibition tests. Serial dilutions were mixed with homologous *E. coli* 0111:B4 antiserum; these mixtures were incubated at 37° for thirty minutes; sheep red blood cells modified by the identical lipopolysaccharide and guinea pig complement were then added. The results are recorded in Table II.

TABLE I.—EFFECT OF ACTIVATED ATTAPULGITE ON *Escherichia coli* LIPOPOLYSACCHARIDE SEROGROUP 0111

<i>E. coli</i> Antiserum	<i>E. coli</i> Lipopolysaccharide, 5 µg./ml., + Activated Attapulgit, µg./ml.						
	5,000	1,700	500	170	50	17	0
1:100	—	—	—	2	3	3	4
1:500	—	—	—	—	2	3	3
1:2,500	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—

— = No hemolysis; 1 to 4 = various degrees of hemolysis.

TABLE II.—EFFECT OF ACTIVATED ATTAPULGITE ON *Escherichia coli* SEROGROUP 0111 LIPOPOLYSACCHARIDE

<i>E. coli</i> Lipopoly- saccharide, μg./0.1 ml.	0111 Lipopolysaccharide, μg./ml.											
	25				5				1			
					Plus Activated Attapulgit, μg./ml.							
	10,000	1,000	100	0	10,000	1,000	100	0	10,000	1,000	100	0
	Hemolysis Inhibition											
0.1	—	—	—	—	4	—	—	—	4	—	—	—
0.05	2	—	—	—	4	—	—	—	4	2	—	—
0.025	3	—	—	—	4	—	—	—	4	3	—	—
0.0125	4	—	—	—	4	±	—	—	4	4	—	—
0.006	4	3	—	—	4	3	2	1	4	4	2	±
0.003	4	4	—	—	4	4	3	3	4	4	3	3
0.0015	4	4	2	3	4	4	3	4	4	4	4	4
0	4	4	4	4	4	4	4	4	4	4	4	4

— = No hemolysis; 1 to 4 = various degrees of hemolysis.

Table II shows that activated attapulgitte either reduces or abolishes the inhibitory effects of lipopolysaccharide. The higher the concentration of attapulgitte and the lower the amounts of lipopolysaccharide used, the greater is the resulting effect. Essentially identical results were obtained in experiments with *E. coli* 0127:B8 and *S. sonnei* lipopolysaccharides as well as with crude antigens.

Experiments were undertaken to determine the effect of activated attapulgitte on the toxicity in mice of endotoxin. For these experiments *E. coli* 0127 B8 lipopolysaccharide (1,000 µg./ml.) was mixed with activated attapulgitte (5,000 µg./ml.) and buffer, respectively. The mixtures were incubated at 37° for one hour and centrifuged. The supernates were used for toxicity determinations by intraperitoneal injection of mice (1 ml. per animal). The results are summarized in Table III.

TABLE III.—EFFECT OF ACTIVATED ATTAPULGITTE ON TOXICITY OF *Escherichia coli* 0127 ENDOTOXIN (LIPOPOLYSACCHARIDE)

Experiment	Groups of Mice Injected with Mixtures of Lipopolysaccharide, 1,000 µg./ml. and			
	Attapulgitte (Activated) 5,000 µg./ml.		Buffer	
	Ratio of Deaths/Number	2 days	6 days	2 days
1	0/12	0/12	3/12	7/12
2	0/11	1/11	1/11	5/11
3	0/6	0/6	2/6	5/6
4	0/12	0/12	9/12	11/2
Total	0/41	0/41	15/41	28/41

Perusal of Table III shows that none of 41 mice injected with attapulgitte treated lipopolysaccharide succumbed, whereas 15 out of 41 control mice died within two days and 28 within six days. The results clearly indicate that activated attapulgitte adsorbs *E. coli* endotoxin as manifested by elimination of toxicity in mice.

DISCUSSION

The present investigation has revealed that activated attapulgitte adsorbs endotoxins (lipopolysaccharides) from enteric bacteria, such as enteropathogenic *E. coli* and *S. sonnei*. It has been established, therefore, that this material adsorbs both exotoxins and endotoxins, the former represented by diphtheria toxin, a protein, and the latter by a lipopolysaccharide complex. Barr (9) reported that activated attapulgitte is not a good adsorbent for enteric bacteria themselves, such as *Salmonella enteritidis* and *Shigella paradyseriae*.

The effects of activated attapulgitte on bacterial endotoxins is of interest from several points of view. In the first place, it is conceivable that such endotoxins play a role in localized intestinal infections. The fact that patients with bacillary dysentery and diarrheal disease due to enteropathogenic *E. coli* develop antibodies against the polysaccharide component of the endotoxin suggests that endotoxin in these illnesses reaches antibody forming cells beyond

the lumen of the intestine. In this connection it should be mentioned that ingestion of "harmless" *E. coli* by human volunteers did not initiate antibody formation against the strain; in contrast, ingestion of enteropathogenic *E. coli* is followed by the appearance of specific antibodies (10). These observations suggest that, because of the very enteropathogenicity of the latter strains, absorption of endotoxin takes place. In the absence of intestinal infection, administration of Cr⁵¹-labeled endotoxin did not result in detectable blood or tissue levels (11). It would be interesting to determine the effect of enteric infection on the absorption of labeled endotoxin. At the present time it is not known whether the endotoxin engendering antibody formation originates from the intestinal contents or from bacteria within or beyond the intestinal wall. If the former were the case, it is conceivable that activated attapulgitte may possess therapeutic value. Only carefully controlled studies in patients will provide the answer to this moot question.

The capacity of activated attapulgitte to adsorb endotoxin is of interest also because of various physiologic disturbances produced by these materials. For a summary the interested reader is referred to the review by Thomas (12). It suffices to mention here that these endotoxins (lipopolysaccharides) profoundly affect the nonspecific resistance to various infections, in part at least via the properdin system (3), contribute to irreversible shock (13), enhance nephrotoxicity of kidney antiscrum (14), and exert antiallergic effects in animals (15).

SUMMARY

Activated attapulgitte adsorbs endotoxins (lipopolysaccharides) from enteric bacteria, namely, enteropathogenic *Escherichia coli* and *Shigella sonnei*. This adsorption was demonstrated by three methods, the enterobacterial hemolysis test, the hemolysis inhibition test, and toxicity studies in mice.

REFERENCES

- (1) Barr, M., and Arnista, E. S., *THIS JOURNAL*, 46, 493 (1957).
- (2) Neter, E., Westphal, O., Lüderitz, O., Gorzynski, E. A., and Eichenberger, E., *J. Immunol.* 76, 277 (1956).
- (3) Springer, G. F., editor, "Transactions of the Second Conference of the Josiah Macy, Jr. Foundation, New York, 1956."
- (4) Neter, E., Bertram, L. F., Zak, D. A., Murdock, M. R., and Arbesman, C. E., *J. Exptl. Med.*, 96, 1 (1952).
- (5) Neter, E., Gorzynski, E. A., Gino, R. M., Westphal, O., and Lüderitz, O., *Can. J. Microbiol.*, 2, 232 (1956).
- (6) Neter, E., and Walker, J., *Am. J. Clin. Pathol.*, 24, 1424 (1954).
- (7) Neter, E., and Gorzynski, E. A., *Proc. Soc. Exptl. Biol. Med.*, 85, 503 (1954).
- (8) Neter, E., Westphal, O., Lüderitz, O., and Gorzynski, E. A., *Ann. N. Y. Acad. Sci.*, 66, 141 (1956).
- (9) Barr, M., *THIS JOURNAL*, 46, 490 (1957).
- (10) Neter, E., Zalewski, N. J., and Ferguson, W. W., *Proc. Soc. Exptl. Biol. Med.*, 82, 215 (1953).
- (11) Sanford, J. P., and Noyes, H. E., *J. Lab. Clin. Med.*, 50, 948 (1957).
- (12) Thomas, L., *Ann. Rev. Physiol.*, 16, 467 (1954).
- (13) Schweinburg, F. B., Davidoff, D., Koven, I. H., and Fine, J., *Proc. Soc. Exptl. Biol. Med.*, 92, 662 (1956).
- (14) Arhelger, R., Smith, F., Brunson, J., Good, R., and Vernier, R., *ibid.*, 96, 424 (1957).
- (15) Meier, R., Bein, H. J., and Jaques, R., *Experientia*, 12, 235 (1956).

A Preliminary Phytochemical Investigation of *Digitalis Lutea* First Year Growth*

By JACK R. COLE† and OLE GISVOLD

The first year leaves of *Digitalis lutea* were extracted by two different techniques, one normal aqueous extraction and one which was enzyme inhibitory. Different results were obtained from each when examined by means of paper chromatography. A system of deacetylation and hydrolysis of the glycosides was developed involving the use of ion exchange resins. Several new solvent systems were developed for the paper chromatographic separation of sapogenins detected.

DIGITALIS LUTEA has been shown to be orally effective to a greater degree than some other members of the digitalis species (1). This fact and other research dealing with the yield of active cardiac glycosides (2-4) of some digitalis species stimulated an interest in the lutea species. Furthermore, since there has been a limited amount of phytochemical research in order to compare the nature of the constituents of the leaves of the consecutive years of growth of the Digitalis species, it was believed a study of this type might produce some interesting results.

The investigation of the second year growth of this plant has been reported (5).

EXPERIMENTAL

Harvesting, Storing, and Extraction Procedures.

—The plants used in this study were grown during the summer of 1955 in the medicinal plant gardens at the University of Minnesota and collected in September, 1955. The storage procedure developed by Hopponen and Gisvold (2) was employed. Fresh leaves also were collected and used. Both lots yielded the same results. The general extraction method of Bay and Gisvold (6) was used to prepare a primary aqueous extract. In some cases emulsions are encountered when this aqueous primary extract is extracted with organic solvents. The use of 15% aqueous alcohol to prepare the primary extract substantially reduced the amount of emulsion that previously had been obtained. The primary extracts after an initial extraction with petroleum ether to remove fatty materials were then successively extracted with the following series of solvents: ether, ether-methylene dichloride 1:3, and methylene dichloride.

The enzyme inhibitory extraction was adapted from that used by Stoll and Kreis (7). Table I, solvent system IV, indicates the chromatographic analysis of the material obtained from this extraction.

Paper Chromatographic Analysis.—The techniques employed by Gisvold and Schwartz (5) were

followed for the most part. Slight modifications in the use of the solvent systems are indicated by the results in Table I.

The authentic samples of the glycosides were obtained from Dr. A. Stoll, Sandoz Co., Switzerland. Digoxigenin was obtained from Dr. Murphy, Analytical Research Laboratories, Burroughs Wellcome & Co., Tuckahoe, N. Y.

The comparison of the crude material obtained from the extraction of the leaves together with reference glycosides using solvent systems I, II, and III indicated the presence of acetyl digitoxin, digitoxin, and acetyl digoxin. In the case of solvent system IV, the presence of lanatoside A was distinctly indicated, and lanatoside B and C weakly present.

Deacetylation and Hydrolysis.—In order further to characterize the glycosides a system of deacetylation and hydrolysis followed by subsequent chromatography was carried out. The usual methods using alkali and acid for these studies are effective, however, the techniques may lead to undesirable changes. Furthermore, exact neutralization is very desirable and inorganic salts are introduced into the sample. In order to overcome some of the objections to the above techniques, ion exchange resins were investigated as a possible substitute for the acid or base.

Deacetylation.—For deacetylation studies, Amberlite resin IRA 400 (Rohm & Haas Co., Philadelphia) was chosen because it is a strongly basic exchange resin. It is a solid polystyrene quaternary amine that is insoluble in most solvents. All reactions should be employed at temperatures below 55-60°, since the resin loses catalytic activity rapidly and irreversibly at slightly higher temperatures. To convert the resin from the chloride form to the hydroxyl form, it is necessary to digest the resin with 1 N sodium hydroxide for approximately twelve hours. The resin is then washed with distilled water until the washings no longer give a basic test with Universal indicator papers. The resin mixture is then filtered and dried in the air.

Approximately 1 Gm. of the dry resin was placed in a test tube and covered with a solution of acetyl digitoxin in methanol (1 mg./cc.). This usually requires approximately 15 cc. of solution. The mixture was sealed and shaken frequently. At regular intervals of one hour, portions of this solution were drawn off and spotted on paper chromatograms. Samples of acetyl digitoxin and digitoxin also were spotted for comparison. The use of ascending chromatography was employed here

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TABLE I.—PAPER CHROMATOGRAPHY RESULTS OF THE CARDIAC GLYCOSIDES

Solvent System in Parts	I			II			III	IV
Methyl isobutyl ketone	5			20			5	..
Toluene	5			10			5	..
Formamide	<i>q. s.</i>			..			<i>q. s.</i>	..
Isopropyl ether	..			5		
Ethylene glycol	..			10		
Butanol			1	..
Ethylacetate	84
Benzene	16
Water	50
	A ^a	D ^b	H ^c	D	H	D	H	A
Development time (hours)	0.5	2.0	1.0	2.5	1.5	2.0	1.0	3.0
Glycoside	<i>R_f</i> Values							
Acetyl digitoxin	0.75	0.77	0.80	0.82	0.75	0.83	0.85	...
Acetyl digoxin	0.20	0.23	0.20	0.31	0.29	0.37	0.35	...
Digitoxin	0.46	0.44	0.46	0.59	0.56	0.56	0.61	...
Digoxin	0.10	0.10	0.09	0.13	0.15	0.24	0.25	...
Digoxigenin	0.34	0.30	...
Ditoxigenin	0.75	0.72	...
Lanatoside A	0.61
Lanatoside B	0.54
Lanatoside C	0.42

^a Ascending. ^b Descending. ^c Horizontal.

as it serves as a rapid method of determining if any deacetylation had taken place. At the end of twelve hours, no reaction had taken place; however, after twenty-four hours some deacetylation had taken place. Substantial deacetylation was obtained after forty-eight hours.

A small column was substituted for a test tube and samples could be drawn off when desired. Deacetylation was obviously very slow and unreliable. When aqueous methanol (90%) was used in lieu of methanol and samples withdrawn every fifteen minutes, progressive deacetylation was obtained that was essentially complete after one hour.

The use of the resin was then applied to the plant extracts. The extract was treated in the same manner as described for the known compounds. A direct comparison was run on the same chromatogram. Acetyl digitoxin disappeared and a new spot, digitoxin, appeared signifying deacetylation had taken place. Solvent systems I, II, and III all were employed in these studies. Horizontal and descending methods were used.

Although the use of sodium hydroxide is more rapid, the use of the resin is certainly a more safe method and removes the danger of any impurities affecting later manipulation of the glycosides.

Ion Exchange Resin Hydrolysis.—For hydrolysis studies, Amberlite IR 120 (Rohm & Haas Co., Philadelphia) was chosen. This resin is a nuclear sulfonic acid type ion exchanger which has unusual stability at high temperatures. Immersion in boiling water appears to have no adverse reaction on the efficiency of the resin. The resin is completely insoluble in water, aliphatic and aromatic hydrocarbons, and most common solvents. It also is extremely stable in strongly acid and alkaline solutions. The resin is supplied in the sodium form and was converted to the hydrogen form by two successive rinses of 10% sulfuric acid. The resin is then washed with distilled water until the washings no longer turn Universal indicator paper acid.

The dry resin was placed in a column and covered with a methanolic solution of the glycoside of the usual concentration (1 mg./cc.). The solution was allowed to stand in contact with the resin for several days but apparently no hydrolysis had taken place. The use of water to speed the rate of hydrolysis was employed here as it had been successful in the deacetylation studies. The use of water did cause reaction to take place but this action was extremely slow. A full twenty-four hour period was required for hydrolysis. The resin-hydrolyzed compounds were run on paper chromatograms with both known aglycones and those produced by acid hydrolyses. They all exhibited the same *R_f* values.

The use of heat was employed in order to increase the rate of hydrolysis. The mixture of resin, digitoxin, and water was refluxed at 70° for fifteen minutes. It was then filtered and the resulting solution was spotted on paper with a known sample of digitoxigenin. Both compounds gave the same *R_f* value. This indicated that with the addition of water and heat the rate of the reaction can be increased quite substantially.

Digoxin was hydrolyzed in the same way but it required thirty minutes at 70° plus the addition of 15% water to cause complete hydrolysis.

Saponins.—The aqueous extracts of the leaves which had previously been extracted to remove the cardiac glycosides were next extracted with *n*-butanol to remove the saponins. Upon concentration of the butanol extract a solid separated that was labeled fraction I. Fraction II was obtained by complete evaporation of the butanol and as could be expected was quite impure. Fractions I and II contained saponins.

Fraction II was extracted with chloroform and a small amount of the material dissolved. A small amount of this chloroform solution was tested by means of the Raymond reaction and a positive cardiac glycoside test was obtained. The chloroform-soluble and chloroform-insoluble materials were recombined and chloroform removed by

TABLE II — PAPER CHROMATOGRAPHIC RESULTS OF THE SAPOGENINS

Solvent Systems in Parts →	A ^a	B	C ^b	D	E	F	G	H	J
Petroleum ether (35-60°)	400	100							
1. Butanol	10	30							
Water	90	70							
Toluene		260		1			3	9	1
n-Butyl ether			q s	1	5	1			
Propylene glycol (stationary phase)			q s	q s	q s	q s	q s	q s	
Methylisobutyl ketone					1				
Petroleum ether (85-100°)						1	1	1	9
Samples	R _f Values —								
Sapogenin I	0.10	0.56	0.54	1.0					
Sapogenin II	0.10	0.55	0.56	1.0					
Tigogenin	0.55	1.0	1.0	1.0	0.08	0.0	0.83	0.81	0.00
Sarsapogenin	0.72	1.0	1.0	1.0	0.98	0.75	0.95	0.95	0.85
Gitogenin	0.09	0.55	0.1	0.61	0.43				
Development time in hours	2	2	2.5	2.5	2.0	2.0	1.5	1.5	1.5

^a Solvent system A and B (Heftmann *et al.*) ascending.^b Solvent system C through J horizontal.

evaporation. This material was then extracted with acetone as acetone dissolves glycosides readily, while saponins are insoluble. The residue remaining after the acetone extraction gave negative cardioteglycoside tests. The acetone extract was evaporated to dryness, purified, and chromatographed by means of solvent system IV. Lanatosides A, B, and C were determined to be present. This indicated incomplete extraction of the cardiac glycosides by the solvents used prior to extraction with butanol.

Paper Chromatography of the Saponins.—The saponins were hydrolyzed by means of hydrochloric acid in alcohol and water mixtures. The reaction mixtures were then extracted with chloroform, dried, and evaporated to dryness and used for chromatography.

The method of Heftmann and Hayden (8) was used for the preliminary chromatography of the saponins. However, since this system requires a twenty-four hour period of equilibration before development, other methods involving solvent equilibration of the paper was sought. The method of equilibrating the paper by means of saturation with organic solvents had been quite successful in the cardiac glycoside series. This method was applied to the chromatography of the saponins. Table II indicates the results of these studies. The standard saponins were obtained from Dr. G. Rosenkranz of Syntex Laboratories, Mexico City.

Solvent systems A and B indicated the presence of tigogenin and gitogenin. C, D, and E separated gitogenin from the other two saponins. F, G, H, and J separated sarsapogenin and tigogenin. H was the most efficient system.

Some difficulty was encountered in these systems from tailing of the compounds. In solvent system C, tailing was appreciable. Solvent systems D, E, and H were practically free from this problem. F showed a small amount of tailing and G showed slightly more tailing, however, the separation was still obvious.

Identification of the Sugar Moities.—Mild hydrolysis of the saponins and subsequent paper chromatographic analysis (3) showed the presence of D-galactose, D-glucose, and D-xylose.

SUMMARY

1. Two extracts of the leaves of *Digitalis lutea* first year growth have been prepared by two different techniques. An enzyme inhibitory technique indicates the presence of the lanatosides A, B, and C. The second method consists of the preparation of aqueous and alcoholic-aqueous extracts and subsequent selective organic solvent extraction of these extracts. In this latter method, some enzymatic degradation has apparently taken place yielding the secondary glycosides.

2. Paper chromatographic analysis was the principal method of qualitative identification.

3. A system of deacetylation and hydrolysis of the glycosides was developed.

4. The saponins of *Digitalis lutea* were extracted with *n*-butanol, hydrolyzed with hydrochloric acid, and analyzed by paper chromatography. Tigogenin and Gitogenin were identified.

5. Some butanol-soluble glycosides of a polar nature not completely extracted previously were found.

6. Several new systems of paper chromatography have been developed for the rapid qualitative identification of the saponins.

7. Hydrolysis of the saponins and paper chromatographic investigation indicated the presence of D-galactose, D-glucose, and D-xylose.

REFERENCES

- (1) White, W. F., *This Journal*, **44**, 607 (1955).
- (2) Hopponen, R. E., and Gisvold, O., *ibid.*, **41**, 146 (1952).
- (3) Appel, R. M., and Gisvold, O., *ibid.*, **43**, 215 (1951).
- (4) Gregg, D. H., and Gisvold, O., *ibid.*, **43**, 106 (1951).
- (5) Schwartz, S. M., and Gisvold, O., *ibid.*, **46**, 324 (1957).
- (6) Bay, G., and Gisvold, O., *ibid.*, **37**, 311 (1948).
- (7) Stoll, A., and Kreis, W., *Helv. Chim. Acta*, **16**, 1049 (1933).
- (8) Heftmann, E., and Hayden, A. L., *J. Biol. Chem.*, **197**, 7 (1952).

Some Physical Chemical Aspects of Suspension Formulation*

By TAKERU HIGUCHI

Difficulties inherent in treating problems associated with formulations of concentrated pharmaceutical suspensions are discussed from the standpoint of a physical chemist. Relationships which determine settling and creaming rates are treated in some detail, a new equation being proposed which may be more valid for hetero-dispersed, irregular particles than the classical Stoke's formula. Factors which influence crystal growth and caking are also discussed. Some reference is also made to diffusional processes of pharmaceutical importance.

A PHYSICAL CHEMIST assigned to problems concerning pharmaceutical suspensions is faced usually with a frustrating situation. His training, first of all, is largely in the basic areas and was designed to help him resolve problems dealing with only a few variables which can be expressed numerically. Unfortunately, pharmaceutical suspensions cannot be physically defined in terms of a limited number of these quantities.

In attempting to describe and define pharmaceutical suspensions, we do use terms expressible as numbers. Density of the internal phase, density of the external phase, phase volume ratio, viscosity of the external phase, etc., can all be described adequately and precisely with numbers. We also express variables such as particle size in terms of numbers but do so with considerable reluctance. The underlying statistics of the method employed, the degree of aggregation, the roughness of the particle surface, etc., strongly influence, in this case, our numerical answer. We make no attempt, on the other hand, to express variables such as size distribution with numbers. The problem furthermore becomes infinitely worse when we try to describe particle shapes precisely in terms of mathematics. This situation severely limits any comprehensive physical chemical approach to the general problem.

The situation, moreover, would not be greatly aided even if these difficult variables were of simpler nature. Unfortunately for physical chemists, we are not faced in these systems with thermodynamic but with kinetic problems. In a system at equilibrium, we are not particularly concerned with the mechanisms by which the end state had been achieved, the end results are always independent of the path taken. In suspension systems the primary concern is the rate of

each physical process. How fast will it flow? What is the rate of settling (or creaming)? How long will it take for the suspended phase to crystallize or to grow? These are problems in kinetics and require reasonable understanding of the actual molecular process by which each phenomenon occurs, an understanding which we are largely lacking at the moment.

For these reasons, solutions to many problems involving pharmaceutical suspension have been obtained largely by the Edisonian trial and error approach. There are, nevertheless, real areas in which the basic, physical chemical thinking can be of major value. In the time available it is impossible to cover these in detail. For this reason only some of the major fields will be listed and a few points indicated to illustrate the nature of the problems involved.

RHEOLOGY

Rheological behavior of suspensions, especially those containing roughly more than 20% by volume of solids, is quite complex. *A priori* theoretical predictions of their flow properties are extremely difficult for reasons stated above. Results of some experimental studies in these areas will be presented by others in this series of papers.

SETTLING AND CREAMING

Difficulties associated with any attempt to relate particle properties and particle concentration to the settling or creaming rate of pharmaceutical suspensions serve as excellent examples of what can be done and what cannot be done with these problems. Let us see first how far simple theories together with simplifying assumptions will take us.

Following Stokes derivation, we can arrive at the familiar expression

$$v = \frac{ds}{dt} = \frac{2}{9} a^2 \frac{(\rho_1 - \rho_2)g}{\eta} \quad (\text{Eq. 1})$$

where the symbols have their usual meaning. In its formulation, we have, in attempting to apply it to real pharmaceutical suspension, assumed the following: (a) Particles are spherical (all of the same shape); (b) particles are all of the same size; (c) particles are sufficiently distant from each other so

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that the movement of one has no influence on its neighbors; (d) the rate is governed solely by hydrodynamic factors.

It is obvious that all of these assumptions are atrociously bad when considering real pharmaceutical systems. Except for the last, each seems worse than the others. The equation has no real value from a quantitative standpoint of predicting settling rates of the relatively concentrated suspensions such as those with which we are usually concerned. Any rigorous attempt to formulate the relationship in absence of these assumptions, on the other hand, seems to be doomed because of the mathematical complexity of the problem.

About ten years ago I considered this problem for relatively concentrated systems from what appeared to be a new approach. I did succeed in obtaining an equation which appears to be based on considerably fewer limitations. This derivation was based on an observation that despite the fact that pharmaceutical suspensions are usually heterodispersed, rather sharp lines of demarcation exist between the particle-free dispersion medium and the relatively concentrated suspension phase for concentrated suspensions which had stood undisturbed for some time. It appeared to me that for such a system the problem can be formulated as a movement of the external liquid phase moving through a bed of the solid internal phase. Fluid flow through packed beds has been treated mathematically by Kozeny and others. Such analyses have led to the Kozeny equation,

$$\mu = \frac{P}{L} \times \frac{1}{k\eta S_r^2} \times \frac{\epsilon^3}{(1-\epsilon)^2} \quad (\text{Eq. 2})$$

where μ is the linear flow velocity, P , the pressure drop across the bed, L , the bed thickness, k , the Kozeny constant approximately equal to 5, η , the viscosity of the permeating fluid, S_r , the specific surface area in sq cm per cm.², and ϵ , the porosity of the bed. There appears to be ample experimental evidence supporting the validity of this equation.

If we assume the relationship to be correct, we can readily derive the rate of creaming (or sedimentation) of suspensions. It can be shown that the pressure drop per unit depth of a uniform suspension acting to move the particles gravitationally is

$$P/L = (1-\epsilon)(\rho_1 - \rho_2)g \quad (\text{Eq. 3})$$

where $1-\epsilon$ is the volume fraction of the internal phase. Substituting this into the Kozeny equation we get

$$\mu = \frac{(\rho_1 - \rho_2)g}{k\eta S_r^2} \times \frac{\epsilon^3}{1-\epsilon} \quad (\text{Eq. 4})$$

For suspensions of uniform spherical particles of radius, a , the equation simplifies to

$$\mu = \frac{(\rho_1 - \rho_2)ga^2}{9k\eta} \times \frac{\epsilon^3}{1-\epsilon} \quad (\text{Eq. 5})$$

These equations are strictly valid only during the initial phases in which ϵ is essentially constant near the moving boundary. It is also assumed in the development of these relationships that there is no change in the composition of the dispersed phase due to differential velocity of small and large par-

ticles. This can be justified partly on the basis that the composition of the dispersed phase near the moving boundary is maintained constant by the fact that small particles swept out of the suspension beyond the boundary would be replaced by particles coming in turn from the interior, and partly because of the caging effect of neighboring particles for these relatively concentrated systems—preventing relative motion of one particle with respect to another.

Comparison of Eqs. 1 and 5 shows a surprising degree of similarity. The factor $\{(\rho_1 - \rho_2)ga^2\}/9\eta$ is common to both. The Stokes law relation contains in addition only the factor 2 whereas the newly derived equation has the additional concentration factor, $\epsilon^3/[k(1-\epsilon)]$. The apparent similarity is somewhat misleading, however, since the physical processes basic to the two equations are quite different. It is obvious that the relationship based on the Kozeny equation only becomes valid for relatively concentrated systems where the suspended phase forms a sort of a bed. The simple Stokes equation on the other hand can only be used where each particle experiences essentially a free fall process.

In Fig. 1 the relative rate of settling for a suspension of uniform spheres of density = 2.0 and radius = 10μ is shown as a function of concentration (per cent phase volume) for Eqs. 1, 5, and for 1 corrected for phase ratio. The rate as predicted by the simple Stokes relationship is independent of the concentration of the internal phase. The equation when modified to correct for the increased flow rate within the bed as the result of decreased effective cross-sectional area of flow path yields a plot which shows a linear decrease in the settling rate with concentration. This plot obviously places an upper limit on the rate of sedimentation. Systems containing more than 10% by volume of the internal phase, according to Eq. 5, offer greater resistance to settling than that predicted by the Stokes equation. For these more concentrated suspensions the rate of settling decreases very rapidly with concentration, being roughly proportional to the fourth power of porosity for ϵ near 0.5.

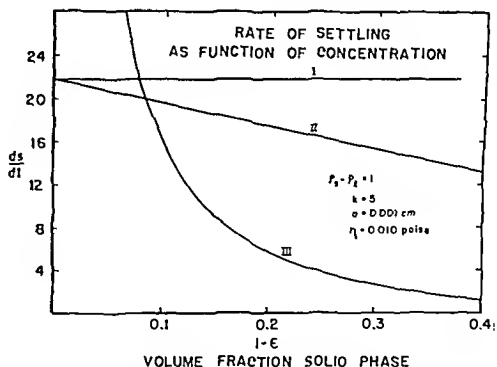


Fig. 1—Relative rates of settling predicted by several relationships are shown. Curve I was calculated directly from the classical Stokes equation. Curve II is the same as I except that it has been corrected for back flow of the external phase. Curve III represents that based on the Kozeny equation as explained in the text.

Although, in the figure, suspensions of uniform spheres were used as examples, the real value of the newly derived relationship is found in Eq. 4 which is for systems containing heterodispersed irregular particles. Since particle size does not occur explicitly in the formula and particle size distribution is not of concern, the equation can be applied directly to any suspensions for which S_r is known. The latter can be determined readily by gas permeability or gas adsorption methods. Unfortunately no real test of the equation, derived has ever been carried out. This has been due to the feeling that the equations only treat the hydrodynamic aspects of the problem and totally neglect the contribution made to suspension stability arising from electrical and other particle-particle interactions. The treatment is thus valid for dispersion, for example, of hard solids in oils but probably is somewhat inadequate for aqueous suspensions of very small particles. This again illustrates the complexity of the problem of suspensions when viewed from theoretical standpoint.¹

CRYSTAL GROWTH AND CAKING OF SEDIMENT

In practice a tendency of a suspension formula to settle or cream slowly does not prevent its adoption. Nearly all commercial products undergo this type of shelf deterioration to varying extents. Normally such suspensions are readily restored to their original states by moderate shaking. In many instances, however, redistribution of the sediment is not so easily effected because of the tendency of some crystals to knit together to form a hard cake. This behavior is often due to growth of the crystalline particles, especially at points of contact.

Increase in crystal size on storage is undesirable for other reasons. Larger particles are difficult to distribute uniformly. They tend to produce gritty texture unsuitable for topical, especially ophthalmic, use. These changes, moreover, result in marked alteration in rheological properties of the products.

The basic phenomenon of crystal growth has received very little attention from serious workers up to the present time, primarily because of the experimental difficulty associated with any attempt to measure the primary process. Although it is always due to growth of the more stable crystal forms at the expense of less stable components, the exact extent of this growth is extremely difficult to measure on real systems. Direct measurement of extent of caking of beds formed by settled suspension particles, moreover, presents even a greater problem. Since the causative process is fairly well established, however, it is possible to postulate from purely physical chemical and mechanistic standpoints some of the factors which may influence the overall process.

Cause.—Growth of a crystalline phase composed of material A is only possible if the thermodynamic activity of A in the solution phase is greater than that of the material in the crystals. The concentration of A, in other words, must be greater in the solu-

tion immediately in contact with the crystal than the solubility of the particular crystal. Such a supersaturated state can be reached by either dissolution of a thermodynamically more energetic form of A into the solution, or cooling of an already saturated solution.

It is evident, therefore, for suspension systems held at a constant temperature to undergo crystal growth that at least part of the suspended phase must be metastable. Since this part of the system would always possess greater solubility, the resulting saturated solutions would be supersaturated with respect to the stable configuration.

Such a metastable condition may arise as a result of a number of different situations. Thus amorphous or glassy preparations always exhibit significantly greater solubility than the corresponding crystalline forms. Most high molecular weight complex organic compounds exhibit polymorphic tendencies, the highest melting form being usually the stable modification. Even stable crystals can be rendered metastable by mechanical treatments which tend to increase the free energy content of these systems. Crushing and grinding operations usually yield particles whose different surfaces exhibit significantly higher or lower solubility. This behavior can be correlated directly with differences in the free surface energy of these powders introduced during the attrition process. Any crystalline flaw produced or an increase in the cumulative length of crystalline edges exposed will produce higher solubility.

As is well known, even perfect crystals differing in size will have different solubilities. Thus, as an example, for the following system: *a powder composed of cubic crystals uniformly 0.1μ along one edge having a density of 2.0, an average interfacial tension of 50 ergs/cm.² and made of substance having a molecular weight of 200*, we can calculate its relative solubility over that of coarse crystals from purely thermodynamic standpoint.

We can write $RT \ln(S/S_0) = \Delta F_s$, where S = solubility in the powder, S_0 = solubility of the gross crystals, and ΔF_s = difference in the molar free energy of those parts of the two systems which are involved in the dissolution equilibria.

This difference can be readily estimated by considering the relative effect on free energy of dissolution of infinitesimal amounts of the solid phase. Since the only energy difference between the large and small crystalline forms is in the surface energy we can derive the relation as follows: Let $A = 6L^2$ and $V = L^3$ where A , V , and L are the area, volume, and lineal dimension of the crystal under consideration. Then $dA = 12LdL$ and $dV = 3L^2dL$. But the corresponding free energy change, $dF = \gamma dA$ = $12L\gamma dL$ and $dF/dV = 4\gamma/L$. The rate of free energy change per mole would then correspond to the rate per unit volume as derived above multiplied by the molar volume of the solute;

$$\frac{dF}{dn} = \frac{M}{P} \frac{dF}{dV} = \frac{4\gamma m}{LP}$$

Introducing the free energy term into the solubility equation we find

$$RT \ln \frac{S}{S_0} = \gamma \left(\frac{1}{L} - \frac{1}{L_0} \right) \frac{4M}{P} \quad (\text{Eq. 6})$$

¹ After completion of this manuscript, the author's attention has been called to a similar treatment by P. G. W. Hawksley, "Some Aspects of Fluid Flow," Arnold, London, 1951. Mr. Hawksley also has utilized the porous bed approach and has obtained good agreement with experimental data.

Substituting in the numerical quantities and neglecting $1/L_0$ since it would be expected to be much smaller than $1/L$, we find $\ln(S/S_0) = 0.08$ at room temperature, or roughly 8% higher solubility for the powder. For crystals tenfold larger, the increase is only 1%; for those only one tenth as large, i. e., 0.01μ on edge, solubility is 2.2 times that of the coarse crystals. It is evident that the effect becomes significant for only very small particles.

Some Possible Approaches to Prevention.—Crystal growth and caking of sediments when due to growth can be largely prevented or reduced in many cases by proper attention to certain fundamentals. The approach can be made either thermodynamically or kinetically.

Since the cause of crystal growth is due largely to the presence of particles at energetic states higher than that of the stable form any and all steps taken which would tend to minimize this energy difference should result in greater stability of the product. Thus selection of the stable crystalline modification in formulating, preparation of powders for suspensions without mechanical comminution, and use of particles of relatively narrow particle size ranges are indicated. Another possibly useful method is based on reducing the free surface energy of the interface between the particle surface and the solvent phase. It is evident from Eq. 6 that if γ were made very small, the effect of particle size could be rendered negligible. It is conceivable that addition of selected surface active agents can readily reduce interfacial tension of most organic drugs against water, for example, to less than 10 dyne/cm.

Even though a suspension system is essentially homogeneous from energetic standpoint, some periodic dissolution and recrystallization may be expected as a result of temperature fluctuations during storage. For these systems and for cases where inclusion of a metastable form cannot be obviated, satisfactory increase in stability can often be achieved by retarding the overall growth process. Rate of both growth and nucleations of the stable form can be reduced, for example, by increasing the viscosity of the suspending medium. Since the kinetics of both processes is largely determined by the diffusion rate, the overall rate would be expected to be approximately an inverse function of viscosity. Thus a tenfold increase in the viscosity of the suspending fluid should result in a corresponding increase in shelf life.

Another solution to the problem makes use again of surface active agents. If a layer of foreign material is adsorbed on the surface of the stable crystalline surface the rate of deposition of solute molecules is greatly decreased. Under certain conditions growth can be totally inhibited even

when in contact with a supersaturated solution. At the same time the rate of solution of the more soluble particles would be correspondingly diminished by the same film. Certain proteins which may undergo some denaturation at the interface seem to be particularly efficacious for this purpose.

DIFFUSIONAL BEHAVIOR OF SUSPENDED DRUGS

This is an extremely interesting area of study for physical chemists as many model situations can be set up and solved mathematically. A good deal of attention has already been directed toward the more basic aspects of the problem and has permitted intelligent application of theories to practical problems.

Since this short survey is hardly adequate to treat the entire field, I have listed below a few types of diffusional problems pertaining to suspensions for which mathematical solutions are presently available.

1. Diffusion of drugs from a finite thickness of a thick suspension layer into a constantly depleted layer, the drug being dissolved in the external phase.
2. Same as 1, except that the drug is now the suspended phase.
3. Diffusion of drug into and through a barrier of finite thickness containing a suspended phase having an affinity for the drug different than the external phase.

Many others of similar type can and have been readily solved.

GENERAL REMARKS

From the preceding examples and discussions it is apparent that physical chemists must choose their ground of operation very carefully if they are to contribute significantly to such applied problems as suspension formulation. As is evident, there are large areas related to the pharmaceutical systems of interest in which we can make good use of our educational background. We must realize, on the one hand, that there are certain aspects concerning which the basic theories are so inadequate that experimentation alone can give us the answer. On the other, we must make the best of unsatisfactory situations. Unlike classical physical chemists we must stand ready to work with messy, complex systems in which many of the variables are not controlled.

The Effect of Vehicle and Particle Size on the Absorption, by the Intramuscular Route, of Procaine Penicillin G Suspensions*

By F. H. BUCKWALTER and H. L. DICKISON

Serum concentrations in rabbits following intramuscular injection of numerous suspensions of procaine penicillin G in various vehicles have been presented. The effects of the nature of the vehicle and the particle size of the procaine penicillin G have been studied. The results indicate that the most practical formulation for delayed absorption is 300,000 units of small particle (less than 5μ) procaine penicillin G per cc. of peanut oil or sesame oil gelled with 2% aluminum monostearate. Extensive data in human subjects are submitted to corroborate the animal results.

A NEW absorption delaying vehicle for penicillin has been reported by Buckwalter and Dickison (1). A formulation using this vehicle and procaine penicillin G of small particle size (particles less than 5μ) has been used for several years as standard treatment for many of the treponemal diseases.

Very little is known about the mechanism of absorption of drugs after any route of administration. Actual determination of the concentration of a drug in the blood stream was virtually unpracticed until the advent of the sulfonamides. Marshall (2) introduced the method for the determination of sulfonamides in blood. With the introduction of penicillin and other antibiotics, the determination of serum concentrations came into widespread use.

These determinations indicated that following intramuscular injections of aqueous solutions of sodium or potassium penicillin G rapid absorption occurred with the production of high serum levels of penicillin. These high serum concentrations decreased rapidly in a few hours due to the rapid excretion of penicillin in the urine after its removal from the site of injection. It became desirable to prevent this rapid absorption with its attendant high serum concentration and to spread the absorption over longer periods of time thus maintaining therapeutic serum concentrations for days instead of hours.

The absorption of penicillin following intramuscular injection could be influenced by the following:

(a) The nature of the penicillin salt used.—In general the more soluble the salt the more quickly it will be absorbed and vice versa. The relatively insoluble salts, procaine penicillin G and benzathine penicillin G give much slower absorption

than the soluble potassium and sodium penicillins.

(b) The nature of the vehicle used.—In general, oil vehicles slow up absorption and aqueous vehicles speed absorption. The field of vehicles for intramuscular use is still virtually unexplored.

(c) Crystal form of the penicillin salt used.—This is rather virgin territory but there does seem to be an effect, which is still undetermined, of crystal form on absorption.

(d) Particle size and particle size distribution.—This is very probably a most important aspect of absorption. Generally large particles are more slowly absorbed than small particles but there are noteworthy exceptions to this.

(e) Coating of the particles of the drug for delaying absorption.

(f) Viscosity of the product.—Viscosity *per se* does not appear to influence absorption since some rather viscous products are absorbed faster than less viscous products.

EXPERIMENTAL

During the work on repository penicillin we compiled a tremendous amount of data on both rabbits and man. This report covers only some of the more salient points and it has been necessary to leave out entirely a good share of the work. As just one example, we can say that zirconium and germanium stearates behave the same as aluminum stearate when gelled with vegetable oils and delay absorption whereas calcium, zinc, and magnesium stearates appear to have very little or no effect on the rate of absorption.

The work presented represents the results obtained following intramuscular injections of various formulations in rabbits and man. In all of the rabbit studies, intramuscular injection of 50,000 units/Kg. were made into the thigh muscles, since we have found that this dosage in rabbits correlates rather closely with the results obtained following the intramuscular injection of 300,000 units into man. In the rabbit studies, a minimum of 5 rabbits was used for each formulation so that all data given represent the average of the results obtained with at least 5 rabbits. Each human subject received a single intramuscular injection of 300,000 units de-

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into the gluteal muscle. In both the animal and human work, we considered 0.03 unit of penicillin per cc of plasma as the theoretical minimum effective concentration. We have found in both rabbits and humans that the percentage of rabbits or humans showing a blood level of 0.03 unit or higher at any stated hour is a most important criterion for a repository product. In many formulations there has been found a good repository effect in a portion of the animals or humans but the percentage was too low for a practical repository product. Many formulations tested showed an average blood level at seventy-two or ninety-six hours of greater than 0.03 unit but the percentage of patients who showed a blood level of 0.03 unit or higher was less than 50%. In this work there was used a requirement or criterion that a good repository product must not only show an average blood level of 0.03 unit or higher but 80% or more of the patients must also show 0.03 unit or higher at whatever hour that might be claimed for the product.

RESULTS

Table I represents a comparison of rabbit blood levels using micronized procaine penicillin G in 11 different vehicles. These vehicles included sesame oil alone, sesame oil gelled with 1 to 5% aluminum monostearate and sesame oil with 1 to 5% beeswax. The procaine penicillin G used had 95% of the particles below 5 μ and essentially 100% of the particles were less than 10 μ . These data indicate that aluminum monostearate has a very considerable effect in delaying absorption whereas beeswax has only a slight effect.

Table II represents a comparison of rabbit blood

levels using 100 mesh procaine penicillin G in the same 11 vehicles described above. The 100 mesh procaine penicillin G represented material which went through an 80-mesh screen but was retained on a 100-mesh screen so that the material had a particle size distribution of approximately 150-175 μ . Microscopic examination corroborated this approximate particle size range. These data indicate that these relatively large particles tend to delay to some extent absorption in the oil alone and in the oil-beeswax vehicles but appear to accelerate absorption in the aluminum monostearate vehicles.

Table III represents a comparison of rabbit blood levels using 250 mesh procaine penicillin G in the same 11 vehicles described above. The 250 mesh procaine penicillin G represented material which went through a 250-mesh screen but was retained on a 325-mesh screen so that the material had a particle size distribution of approximately 45-60 μ . Microscopic examination corroborated this approximate particle size range. These data indicate that these relatively small particles are absorbed rather rapidly in the oil alone and in the oil-beeswax vehicles but are absorbed rather slowly from the aluminum monostearate vehicles.

The entire series represented in Tables I, II, and III were repeated using peanut oil in place of sesame oil. The results obtained were quite similar to those obtained with sesame oil. There is apparently no significant difference between the two oils in so far as absorption is concerned.

Table IV represents a condensation of some of the results in Tables I, II, and III. It shows the effect of particle size of the procaine penicillin G in sesame oil, in sesame oil gelled with 2% aluminum monostearate, and in sesame oil containing 5% beeswax. These data indicate that large particles of procaine

TABLE I.—PENICILLIN SERUM CONCENTRATIONS IN RABBITS.
MICRONIZED PROCAINE PENICILLIN G (300,000 UNITS/CC) IN SESAME OIL VEHICLES

Vehicle	Normal	Average Blood Levels, Hours											
		1	1	21	48	72	96	120	144	168	192	216	240
Sesame Oil	NR	5.53	2.27	0.03	NR								
+ 1% ALMST ^a	NR	0.81	0.83	0.51	0.15	0.35	0.07	0.02	NR				
+ 2% ALMST	NR	1.03	0.71	0.33	0.26	0.10	0.09	0.05	0.01	0.06	0.02	NR	
+ 3% ALMST	NR	3.39	1.52	1.03	0.69	0.20	0.13		0.02	0.08	0.02	NR	
+ 4% ALMST	NR	1.53	2.16	0.87	0.28	0.11	0.04	0.08	0.08	0.06	NR		
+ 5% ALMST	NR	1.26	1.04	0.56	0.07	0.20	0.03	0.05	0.02	0.015	0.03	0.02	NR
+ 1% Beeswax	NR	1.93	1.78	0.33	0.10	NR							
+ 2% Beeswax	NR	2.18	1.78	0.38	0.07	0.07	0.02	NR					
+ 3% Beeswax	NR	2.36	1.58	0.11	0.06	NR							
+ 4% Beeswax	NR	1.35	1.76	0.35	0.01	NR							
+ 5% Beeswax	NR	1.11	2.28	0.28	0.07	NR							

^a ALMST = Aluminum monostearate. NR = No zones of inhibition.

TABLE II. PENICILLIN SERUM CONCENTRATIONS IN RABBITS
100 MESH (LARGE PARTICLE) PROCAINE PENICILLIN G (300,000 UNITS/CC) IN SESAME OIL VEHICLES

Vehicle	Normal	Average Blood Levels, Hours											
		1	1	21	48	72	96	120	144	168	192	216	240
Sesame Oil	NR	3.50	1.45	0.16	0.05	NR							
+ 1% ALMST	NR	2.03	1.66	0.23	0.09	0.04	0.02	0.008	NR				
+ 2% ALMST	NR	2.58	1.83	0.21	0.04	0.01	NR	0.04	NR				
+ 3% ALMST	NR	2.21	1.27	0.21	0.11	0.16	0.11	0.01	0.01	0.02	NR		0.02
+ 4% ALMST	NR	1.90	1.01			0.14	0.16	0.05	0.03	0.03			0.11
+ 5% ALMST	NR	1.88	1.05			0.15	0.08	0.05	0.03	0.02			0.01
+ 1% Beeswax	NR	4.13	2.58	0.015	NR	NR							
+ 2% Beeswax	NR	5.58	4.13	0.15	0.01	0.008	NR						
+ 3% Beeswax	NR	2.61	1.92	0.17	0.04	0.008	NR						
+ 4% Beeswax	NR	3.71	2.68	0.10	0.03	NR	0.01	NR					
+ 5% Beeswax	NR	3.66	2.84	0.11	0.05	0.008	NR	NR					

